Medical Library MAR 10 1928

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

MOVEMENTS OF THE STOMACH. *Walter C. Alvarez and Arnold Zimmermann* 261

A METHOD OF OBTAINING ALVEOLAR AIR FROM NORMAL DOGS AND A COMPARISON OF THE CARBON DIOXIDE TENSIONS OF ALVEOLAR AIR AND ARTERIAL BLOOD. *Ralph G. Smith and Peter Heinbecker* 271

THE EFFECT OF INJECTION OF FOLLICULAR EXTRACT ON THE SEX ORGANS IN THE GUINEA PIG AND THE INTERACTION BETWEEN THE FOLLICULAR SUBSTANCES AND SUBSTANCES GIVEN OFF BY THE CORPUS LUTEUM. *Leo Loeb and W. B. Kountz* 283

THE HEMODYNAMIC ACTION OF MINIMAL EFFECTIVE DOSES OF EPINEPHRIN IN THE UNANESTHETIZED DOG. *Carl A. Dragstedt, Ardath H. Wightman and John W. Huffman* 307

FEEDING EXPERIMENTS WITH PLANTS AT DIFFERENT STAGES OF DEVELOPMENT. III. SYNTHESIS OF VITAMIN IN CORN. *Maxwell Karshan, Frances Krasnow and Benjamin Harrow* 314

MOVEMENTS OF THE CECUM. *Walter C. Alvarez and Arnold Zimmermann* 317

STUDIES ON VIGOR. XV. THE EFFECT OF THYROIDECTOMY ON SPONTANEOUS ACTIVITY IN THE RAT, WITH A CONSIDERATION OF THE RELATION OF THE BASAL METABOLISM TO SPONTANEOUS ACTIVITY. *M. O. Lee and E. F. Van Buskirk* 321

EFFECTS OF THYMUS, MUSCLE AND PITUITARY EXTRACTS ON NORMAL AND THYRO-PARATHYROIDECTOMIZED DOGS. *E. Larson and N. F. Fisher* 330

SIMULTANEOUS DETERMINATION OF THE PULMONARY AND SYSTEMIC CIRCULATION TIMES IN MAN AND OF A FIGURE RELATED TO THE CARDIAC OUTPUT. *W. F. Hamilton, John Walker Moore, J. M. Kinsman and R. G. Spurling* 338

XVIII. THE DEPRESSOR EFFECT OF EPINEPHRIN IN DECEREBRATED ANIMALS. *Charles M. Gruber* 345

CHEMICAL CHANGES IN THE BLOOD DURING FASTING AND SUBSEQUENT REFEEDING. II. INORGANIC COMPONENTS. *Sergius Morgulis. With the technical assistance of V. L. Bollman* 350

THE PHOTODYNAMIC ACTION OF HEMATOPORPHYRIN. *Ethelberta Norris, Rask and W. H. Howell* 363

CALCIUM AND ULTRA VIOLET IRRADIATION. A. THE EFFECT OF ULTRA VIOLET RADIATION UPON SERUM CALCIUM. B. CALCIUM UTILIZATION ON A CALCIUM-POOR DIET WITH ULTRA VIOLET RADIATION. *Lawrence T. Fairhall* 378

STUDIES ON THE PHYSIOLOGY OF SLEEP. V. SOME EXPERIMENTS ON PUPPIES. *Nathaniel Kleitman* 386

THE ACTION OF MYRTILLIN IN PANCREATIC INSUFFICIENCY. *Leonard Benjamin Shpiner* 396

THE PHOSPHATE CONTENT OF RENAL CAPSULAR FLUID IN NECTURUS. *Francis O. Schmitt and H. L. White* 401

A STUDY ON THE MECHANISM OF THE STROPHANTHIN MYDRIASIS IN THE RAT. *Robert Dane Barnard* 407

THE EFFECT OF LOW PROTEIN AND PROTEIN-FREE DIETS AND STARVATION ON THE VOLUNTARY ACTIVITY OF THE ALBINO RAT. *F. A. Hitchcock* 410

THE RELATION BETWEEN THE THRESHOLD OF NERVE RESPONSE AND POLARIZATION BY GALVANIC CURRENT STIMULI. *George H. Bishop* 417

POLARIZING EFFECTS OF ELECTRICAL STIMULI UPON CONTRACTION OF SKELETAL MUSCLE. *John M. McCaughan and George H. Bishop* 437

THE EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT. V. THE OFFSPRING. *James Rollin Slonaker* 442

(Contents continued on cover 4)

VOL. LXXXIV—No. 2

Issued March 1, 1928

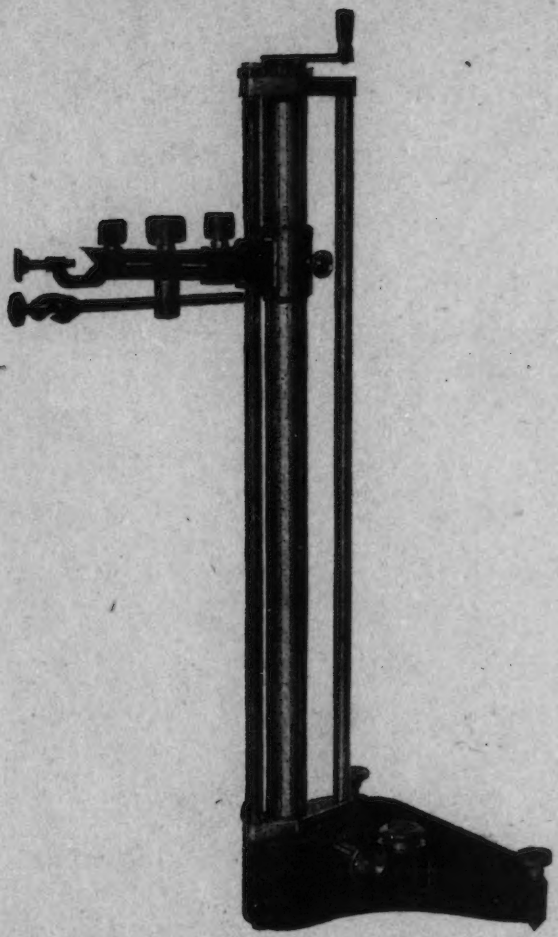
BALTIMORE, U. S. A.

1928

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

HEAVY UNIVERSAL STAND



THE heavy cast iron base makes this stand unusually rigid. A spirit level is fixed to the base so that the supporting rods may be brought quickly into the vertical position by adjusting the leveling feet of the stand. The arm of the instrument is raised and lowered by means of a spindle activated by a crank on the top of the supporting tube. This arm is equipped with two clamps; the upper one is adjustable through all angles in a horizontal plane, and it can hold also a vertical rod; the lower is adjustable to all positions. A rod of the same length as the spindle affords additional opportunity for supporting apparatus. The entire vertical portion of the stand can be rotated through an angle of 360° by means of a milled screw head. More rapid adjustments can be made by throwing the worm and gear out of contact, when the supporting stand becomes freely movable.

Price $75.00

JOSEPH BECKER 437 West 59th St., New York

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 84 MARCH 1, 1928 No. 2

MOVEMENTS OF THE STOMACH

WALTER C. ALVAREZ AND ARNOLD ZIMMERMANN

From the Division of Medicine, Mayo Clinic and The Mayo Foundation, Rochester, Minnesota

Received for publication August 8, 1927

It is remarkable that after twenty years of fluoroscopic study of the stomach, experts are not agreed as to the exact mode of progress of the peristaltic wave over the pars pylorica. Older physiologists, influenced largely by the observations of Hofmeister and Schütz (1885) on the excised stomachs of dogs, taught that the contraction traveled as a wave until it reached the prepyloric ring; that there a band of muscle contracted so powerfully as almost to divide the stomach into two pouches, and that then the pars pylorica contracted systolically much like the ventricle of the heart. Later, with the introduction of the barium meal and the fluoroscope, this conception was abandoned, and its place taken by Cannon's idea of a wave traveling peristaltically all the way to the pylorus.

Cannon (1911) did not see the deep constrictions at the prepyloric ring described by Hofmeister and Schütz, and only once saw the concentric type of contraction in the pars pylorica. Kästle, Rieder and Rosenthal (1909), who first studied serial roentgenograms of the stomach of man, described the waves as traveling to the pylorus, but Groedel (1912), who used the same technic, thought that this type of activity was exceptional, and that as a rule the peristaltic contractions changed into systoles a few centimeters from the pylorus.

A number of writers, among whom may be mentioned Cole (1911), Wheelon and Thomas (1920), M'Crea, M'Swiney, Morrison and Stopford (1924), Klein (1926) and Krzywanek (1927), have since studied movements of the stomach and have come to see that there are many types of peristalsis in different animals and even in the same animal at different times. A fairly complete review of the literature is given by Alvarez (1928).

The first to record many of these peculiar types of peristalsis graphically

were Alvarez and Mahoney (1921) and Alvarez and Zimmermann (1926). There is still great need, however, for exact information as to the place of origin and mode of conduction of the peristaltic wave over the stomach.

The heart cycle repeats itself with such regularity that Lewis, with a double electrocardiograph, was able to analyze the progress of the wave centimeter by centimeter as it spreads over the wall of the auricle and down onto the ventricle, but this cannot be done in the stomach because there the cycle varies from moment to moment; several waves are often traveling at one time, and the rate and mode of conduction are not always the same. As a result one cannot easily trace the progress of a wave through a series of records made simultaneously in different regions. If the rate of conduction were constant one could take a record from the middle portion of the stomach, move it a short distance to the right, and superimpose it on another record made from some region caudad but, as it is, if this is attempted many peaks and troughs do not coincide, and it is hard to identify parts of any one peristaltic wave; in fact one cannot always be sure that a traveling wave passed. As if this were not sufficiently confusing, many small contractions of local origin are found superimposed on the traveling waves and at times, as a wave is moving down the stomach, another will appear a little ahead of it and then go on to the pylorus.

In the face of all these difficulties it seemed as if the problems of gastric motility could be solved only with the help of a large number of records made simultaneously from different parts of the stomach. After many experiments with different types of recording devices, it was finally decided that the simplest and best way in which to get the desired records would be to take motion pictures, and that is what we have been doing for the last year and a half. The animal is opened as usual under a bath of salt solution and bits of black thread are placed here and there under the serous coat to serve as markers. In the earlier experiments serrefines or Michel clamps were used. When the film is developed, successive pictures are thrown on a screen and the distances between the markers are measured and plotted as ordinates with intervals of time as abscissas.

If the motion being recorded is fast, every picture can be used and the abscissas will represent one-sixteenth of a second. Ordinarily, with the stomach, it is sufficient to measure every eighth or sixteenth picture. The ordinates are measured downward from a base line so that a rise in the resultant curve will represent shortening just as it does in the usual curves obtained with heart levers and a smoked drum. In the final figure the base lines are either erased or the curves are redrawn in proper sequence on another piece of coördinate paper.

We realize that owing to the curvature of the ventral surface of the stomach and to a little rotation that sometimes takes place, a small error enters into some of the measurements, particularly in the pars pylorica,

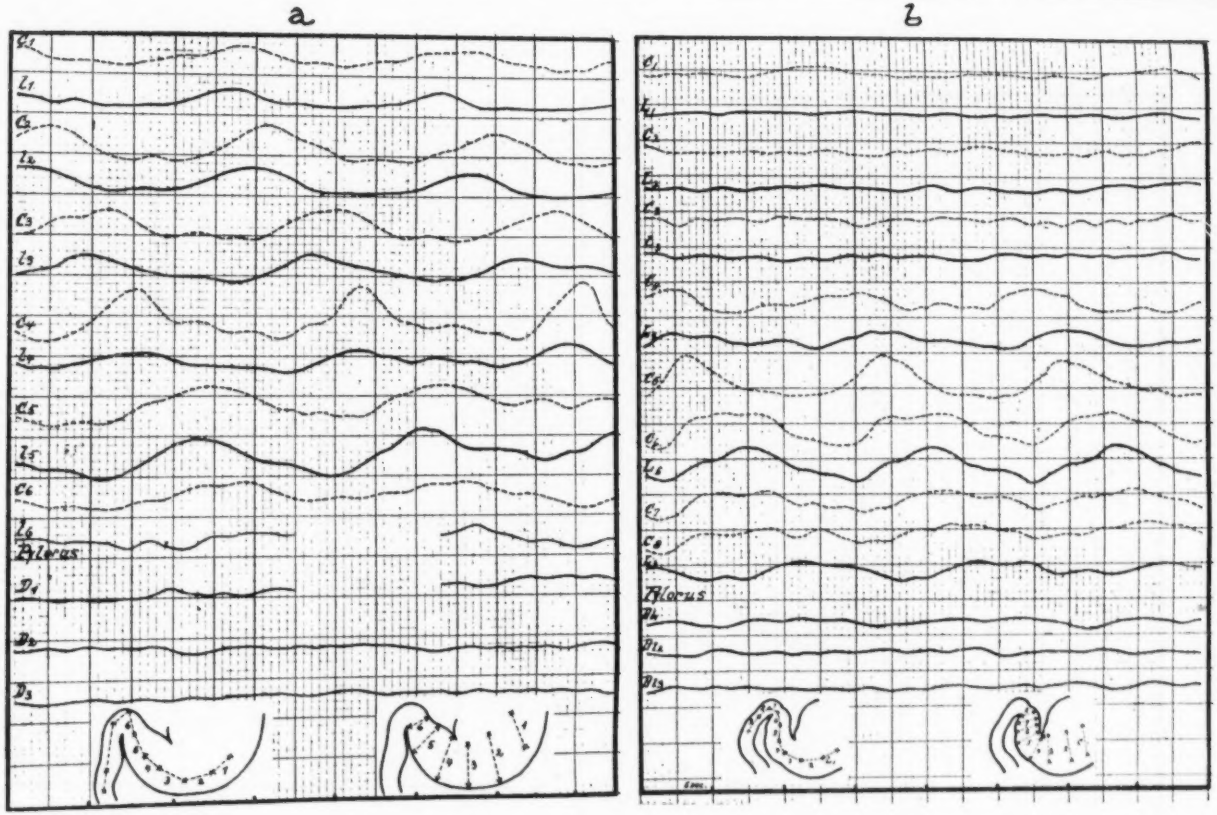


Fig. 1. Records from the stomachs of dogs. The small diagrams show the position of markers on the stomach, and the numbers correspond to those on the curves. *C4* in *a* thus represents the activities of the circular muscle at the level of the incisura. *D1*, *D2*, and *D3* show the movements of longitudinal muscle in the duodenum. The time record marks intervals of five seconds. A gap in the record is due to the temporary disappearance from view of a few markers.

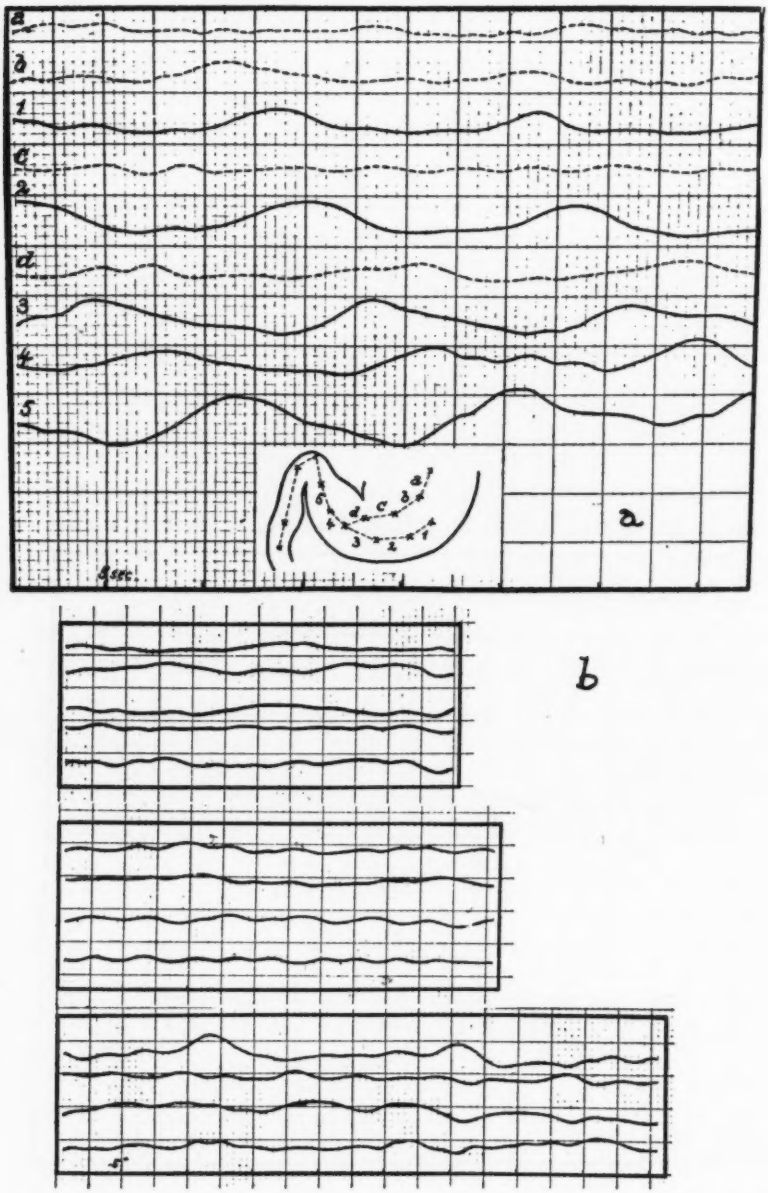


Fig. 2. *a*. Records from a dog's stomach showing momentary differences in the rate of conduction of waves. *b*. Three sets of records of the small, rhythmic, sometimes segmenting movements in adjacent areas in the fundus of the stomachs of dogs.

but for the present this is not disturbing as we are now concerned with the detection and analysis of movement and not with the problem of amplitude.

The most satisfactory animal used so far has been the dog, as some peristalsis has been seen in almost every one opened. This cannot be said of the cats and rabbits. The anesthetic given to the dogs has generally been morphine and ether, and for the cats and rabbits urethane (by rectum). In some of the animals choline was given to stimulate peristalsis.

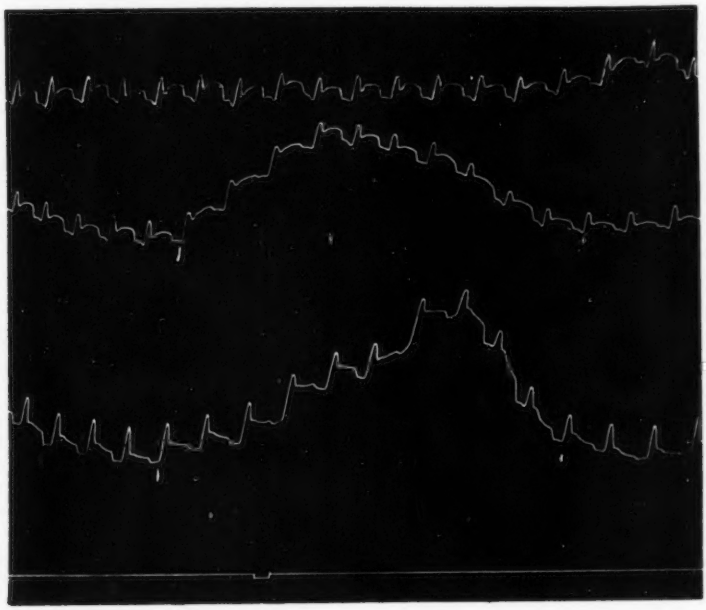


Fig. 3. The passage of a wave over the pars pylorica of a rabbit. Threads running to heart levers were fastened first, to the lower part of the corpus ventriculi; second, to the pre-antrum, and third, to the pars pylorica. Dots mark synchronous points. The small movements are respiratory in origin. The muscle at two levels begins to contract simultaneously but the peak of the lower curve comes so much later than that of the upper that it looks as if a peristaltic wave had passed.

RESULTS. When we came to analyze the records of gastric peristalsis secured with this technic (figs. 1 and 2) we no longer wondered at the disagreement between the various workers in the past because it would be hard to imagine a phenomenon more cunningly designed to deceive an observer. As will be seen in figure 1, while the wave in the longitudinal muscle runs peristaltically all the way to the pyloric line, the wave in the

circular muscle changes into a systole, or perhaps we should say simply that the region next to the spincter contracts ahead of time. This premature contraction was observed years ago by Alvarez who thought that it was due to a shorter latent period of the muscle in this region (1916).

As if this difference between the activities of the circular and longitudinal muscle were not sufficiently confusing, another peculiar feature is added. As can be seen from some of the records, the circular muscle next to the pylorus begins to contract at the same time as the segment just above, but it shortens so much more slowly that if one were to look only at the peaks of the curves one would get the impression that a peristaltic wave had passed. This is seen best in the mechanical record shown in figure 3, probably because there the movements of the writing point represent to some extent an integration of the movements of two muscular coats.

We now have photographic records of scores of gastric waves in dogs, and so far they all show some sign of the systolic type of contraction of the circular fibers of the last centimeter or two of the stomach. The amount of pars pylorica involved in the systole varies, however, not only in different animals but from time to time in the same animal. In one dog we saw the marked contraction of the transverse band described by the older physiologists (fig. 4, first two pictures on the right). Generally this contraction was part of a peristaltic wave which passed down over the pars pylorica, but occasionally it was followed by a systolic narrowing of the whole pyloric third of the stomach, such as is depicted in the third picture in figure 4.

We suspect that the way in which a gastric wave finishes its course in the antrum has much to do with the success or failure that attends its efforts to push material into the duodenum. Some of the systoles are well designed to force food through the pylorus while others seem clearly to block the descending wave and to close the sphincter in its face.

The new records show again the dissociation between the activities of the fundus and pars pylorica observed by Alvarez and Mahoney in electrogastrograms, and they show the small waves of local origin superimposed on the larger peristaltic waves (fig. 1 *b*).

One of the most interesting observations made during this study is that the small contractions in the fundus, described by Alvarez and Zimmermann (1926), do not advance toward the pylorus but appear, now here, now there, much as do the rhythmic segmenting movements of the small bowel (fig. 2 *b*).

The analysis of motion pictures of the movements of the dog's stomach confirms another observation made by Alvarez (1927) on rabbits, that the contractions of the lower portion of the pars pylorica sometimes resemble those of the duodenum more than those of the stomach (fig. 1 *b*). It is

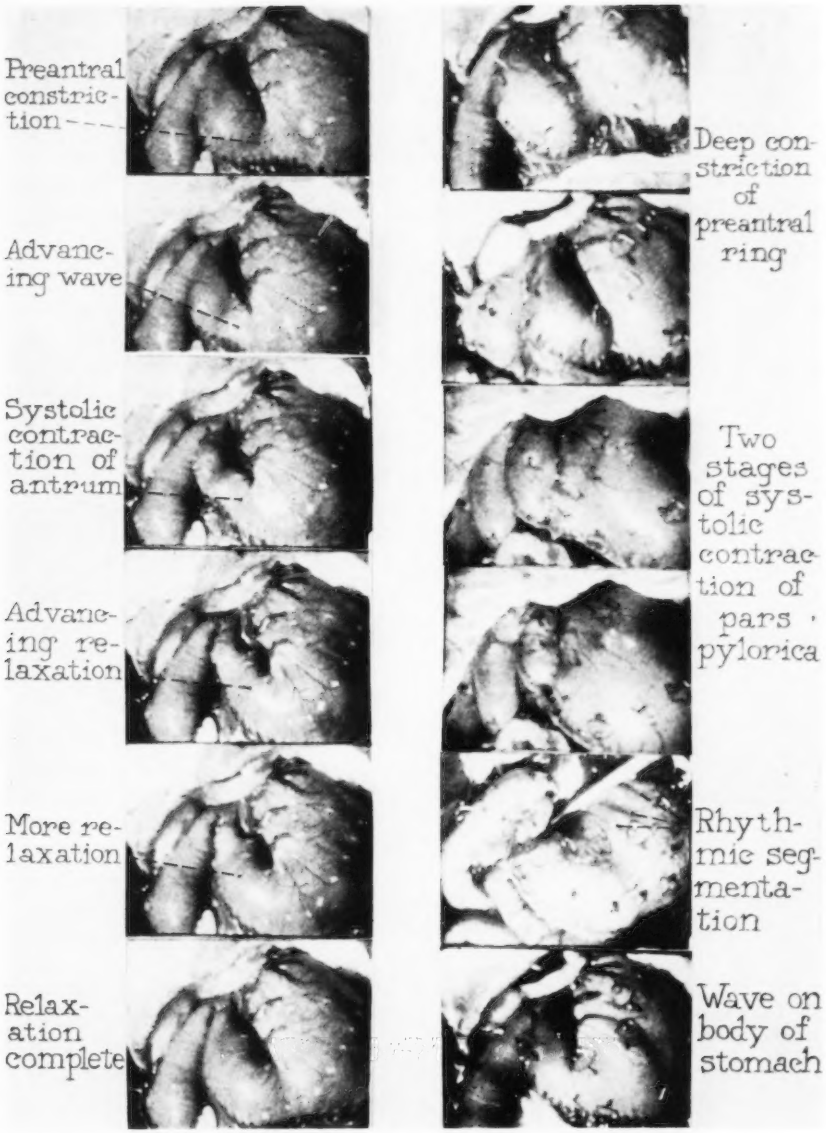


Fig. 4. Enlargements from Ciné motion picture film showing the progress of waves of contraction and relaxation over the stomach, a systole of the pars pylorica, deep constrictions of the pre-antral ring, rhythmic segmentation near the cardia, and a wave performing the maneuver of column left.

probable that at times some rhythmic stimulus spreads backward through the pyloric block, but this explanation is not always necessary because the muscle of the stomach often contracts rhythmically with a rate even faster than that of the duodenum (Alvarez and Zimmermann, 1926).

Another curious form of activity that we hope to study further is the wave of relaxation that seems often to follow in the wake of contraction. It is shown in the fourth, fifth and sixth pictures in figure 4. At times the study of this wave is made difficult because a contraction near the incisura apparently forces air or other material into the pars pylorica and thus distends it passively. Another interesting observation is that the longitudinal and circular muscular coats of the stomach at times contract independently.

We have found it easy in the dog to produce waves running orad over the lower half of the stomach simply by pinching the pylorus. These waves break against the descending ones and disappear. Occasionally we have seen ripples running orad over the body of the stomach while at the same time deep waves were running normally over the pars pylorica.

Many measurements will have to be made on the records before we can say much about the rate of conduction. In one dog it was found to decrease progressively from about a centimeter a second in the body of the stomach to about half a centimeter a second, near the pylorus. In other animals the impression gained from watching the waves was that they slow up at the incisura and then sweep more rapidly again over the pars pylorica. The rate appears to be considerably faster on the greater curvature than on the lesser because the left flank of the wave has to travel so much farther than the right in order to reach the pylorus on time. This is apparent in the last picture in figure 4. The point is an important one, and has to be kept in mind by the surgeon whenever he exaggerates the disparity by excising any part of the lesser curvature. He then has to make a grastro-enterostomy because otherwise the stomach will not empty.

While studying our mechanical records we came to the conclusion that the rate of conduction varies from moment to moment, and that impression is supported by records such as those presented in figure 2 *a*. There it can be seen that the right flank of a wave reached segment *b* ahead of the left flank at *1*, but later, when the next wave passed, conduction had become so much better in the region of the greater curvature that the two flanks were abreast. Still more surprising, the right flank practically disappeared a centimeter below *c* while the left shot ahead almost instantly to *2*, and then advanced more slowly through segments *3*, *4* and *5*.

A question that has bothered us is: Are these peculiar types of peristalsis that we have seen in the stomach ever present in the normal intact animal or are they due purely to the abnormal conditions of the experiment? It may well be that some of them are brought out by the opening

of the abdomen but we know that they are not all due to that because, during the last fifteen years, one of us (Alvarez) has seen most of these types of contraction in the stomachs of men and women examined under the roentgenoscope. Some of them have been described also by Auer (1908), who did nothing more to his rabbits than to shave the ventral surface and watch the waves rippling under the thin covering of muscle and skin, and by Borchers (1921), who used cats in which a celluloid window was incorporated into the abdominal wall.

SUMMARY

Authorities do not yet agree about the mode of travel of the waves over the stomach. The reason is that it has not been easy to record simultaneously the movements of enough areas on the gastric wall. Gastric activity is now being studied with the help of motion pictures. Curves made by plotting, from successive pictures, the distances between markers on the surface of the stomach show that there are many types of gastric activity: small, shallow, more or less stationary contractions producing what looks like rhythmic segmentation, tonus changes, peristaltic waves running to the pylorus, and peristaltic waves changing into systoles of some or all of the pars pylorica; peristaltic waves that stop at some point, usually at the pre-antral line; waves that begin at the pre-antral line, contractions of local origin superimposed on traveling ones, waves of relaxation, and occasional waves of reverse peristalsis.

The problem of analyzing the movements in the lower third of the stomach is made difficult by the fact that while a wave is traveling peristaltically to the pylorus in the longitudinal muscle, the circular muscle contracts systolically. The systoles seen in this study were seldom so marked as those described by the early physiologists. There is not much sign of a sphincter antri such as they described, but at times the waves in the region of the incisura are deep enough to divide the stomach momentarily into two pouches.

Gastric peristalsis is a more complex phenomenon than has been thought in the past, and some of the forms of activity here described may perhaps be associated with discomfort and symptoms of indigestion.

BIBLIOGRAPHY

ALVAREZ, W. C. 1916. This Journal, xl, 585; xli, 321.
1922. Journ. Amer. Med. Assoc., lxxviii, 1116; lxxix, 1281.
1923. Amer. Journ. Roentgenol., x, 31.
1927. Journ. Amer. Med. Assoc., lxxxviii, 472.
1928. The mechanics of the digestive tract. New York, Hoeber, 2nd ed.
ALVAREZ, W. C. AND L. J. MAHONEY. 1921. This Journal, lviii, 476.
1923. This Journal, lxiv, 371.

ALVAREZ, W. C. AND A. R. ZIMMERMANN. 1926. This Journal, lxxviii, 405.
AUER, J. 1908. This Journal, xxiii, 165.
BORCHERS, E. 1921. Deutsch. Zeitschr. f. Chir., clxii, 19.
CANNON, W. B. 1911. The mechanical factors of digestion. New York, Longmans.
CHILD, C. M. 1915. Senescence and rejuvenescence. Chicago, The University of Chicago Press.
COLE, L. G. 1911. Arch. Roentg. Ray, xvi, 242.
GROEDEL, F. M. 1912. Die Magenbewegungen. Hamburg, L. Gräfe and Sillem.
HOFMEISTER, F. AND E. SCHÜTZ. 1885. Arch. f. exper. Path. u. Pharm., xx, 1.
KAESTLE, C. 1918–1919. Fortschr. a. d. Geb. d. Röntgenstrahlen, xxvi, 181.
KÄSTLE, C., H. RIEDER AND J. ROSENTHAL. 1909. München. med. Wochenschr., lvi, 280.
KLEIN, E. 1926. Arch. Surg., xii, 571, 583, 1224.
KRZYWANEK, F. W. 1927. Pflüger's Arch., ccxv, 481.
M'CREA, E. D., B. A. M'SWINEY, J. W. MORRISON AND J. S. B. STOPFORD. 1924. Quart. Journ. Exper. Physiol., xiv, 379.
PALUGYAY, J. 1921. Pflüger's Arch., clxxxvii, 232.
REYNOLDS, L. AND C. W. MCCLURE. 1922. Arch. Int. Med., xxix, 1.
THEILE, P. 1917. Zeitschr. f. Kinderheilk., xv, 152.
WHEELON, S. AND J. E. THOMAS. 1920. Journ. Lab. Clin. Med., vi, 124.

A METHOD OF OBTAINING ALVEOLAR AIR FROM NORMAL DOGS AND A COMPARISON OF THE CARBON DIOXIDE TENSIONS OF ALVEOLAR AIR AND ARTERIAL BLOOD

RALPH G. SMITH AND PETER HEINBECKER[1]

From Departments of Pharmacology and Physiology, Washington University, School of Medicine, St. Louis

Received for publication October 22, 1927

The purpose of this paper is to describe a method of obtaining alveolar air from normal unanesthetized dogs and to report a series of experiments showing the degree of equilibrium which exists between the carbon dioxide content of arterial blood and the air so obtained. The alveolar air was collected synchronously with blood drawn from the left ventricle, the experiments being carried out on resting dogs. The blood then was analyzed for oxygen and carbon dioxide. With the same sample of blood three points were determined on the carbon dioxide dissociation curve. By means of the curve thus drawn, the degree of equilibrium between the carbon dioxide of the air obtained and the arterial blood, was ascertained in the usual manner. In this series of experiments four dogs were used and from six to ten experiments were carried out on each dog, after the technique had been perfected. From the results the normal variation of the dissociation curve in individual dogs was ascertained and the variation found in various dogs was indicated.

METHOD OF OBTAINING ALVEOLAR AIR. The general plan of the method consists in the training of the dog to lie quietly for the requisite period of time wearing a closely fitting plaster of Paris mask similar to those described by Marshall (1926). To the mask is connected a valve devised to collect a portion of the air at the end of a normal expiration thus following the principle of Krogh and Lindhard (1914).

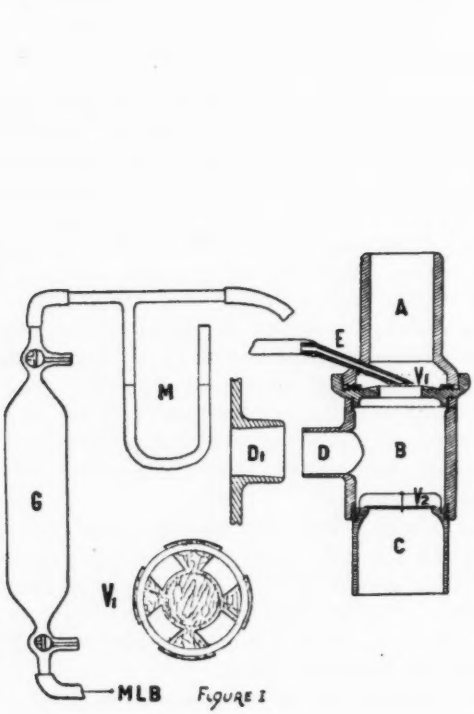
It resembles the valve described by Henderson and Haggard (1925) in that a portion of air last expired is automatically collected.

An anesthetic is administered for the construction of the mask which is made by winding plaster of Paris bandages closely around the muzzle of the dog. Into the end of the mask over the nostrils is incorporated a short metal tube for connection to the valve. Over the posterior opening of the mask are stretched two overlapping pieces of light weight rubber

[1] Fellows in Medicine of The National Research Council.

dam which at the time of use are thickly coated with petrolatum. This arrangement forms an air tight fitting for ordinary respiratory pressures. The mask is held in place by three linen straps secured together behind the head.

The brass valve may be most readily described by referring to figure 1. It consists of three sections of which A and C are made to screw into the middle section B which communicates with the mask through D, the latter being made to fit D_1 of the mask by means of a conical joint. At the openings between B and the two sections A and C are two valves V_1 and



V_2 respectively. V_2 is a mica valve riding on a brass collar in the upper part of C and permitting air to pass inward. V_1 is a valve made of pliable parchment such as is used by Prof. A. Krogh in his metabolism apparatus and which he kindly supplied. It is cut as shown in cross-section in figure 1. The intact central portion is sufficiently large to cover the opening between B and A sealing over a brass rim about 0.5 mm. in height. In the outer part of the membrane is cut a series of holes sufficient in area

that they cause no resistance to the passage of air. The valve is held in place by a brass ring about 1 mm. in width which rests on its outer edge and which in turn is held in place by section *A* which screws down on the ring. The small tube *E* through which the alveolar air is collected is soldered into section *A* and is made so that the lower opening is 0.5 mm. above the level of the rim over which valve V_1 seals. Adjustments of the level of *E* in relation to the valve may be made during use by screwing section *A* upward or downward in *B*.

The sequence of events during a respiratory cycle is as follows. On inspiration valve V_2 is opened and air passes inward through *C*, *B* and *D*, valve V_1 being closed by the negative pressure in *B* and the end of *E* being open. On expiration valve V_2 closes and V_1 opens, at the same time sealing off the mouth of *E*. Thus the expired air passes outward through *D*, *B* and *A* which may be connected with a spirometer if so desired. Consequently during expiration when the expired air is passing upward through *A*, *E* is sealed off by valve V_1. During inspiration the end of *E* is open to the air in *A*, which is the last air expired. If *A* is not connected with a spirometer a piece of wide bore glass tubing one to two feet in length is attached to it, in order to prevent the diffusion of atmospheric air inward during inspiration.

The alveolar air is collected by mercury displacement in a glass tube of 20 to 25 cc. capacity fitted with three-way stopcocks and connected with a mercury levelling bulb, *MLB*, as indicated in figure 1. In this way it differs from the method used by Henderson and Haggard (1925) in which inspiration is the motive force. In the dog the resistance to inspiration necessary to permit sampling by this force acting through a valve was shown at the outset to be so great as not to permit its use. It is highly essential that the apparatus in experiments of this type does not modify the type of respiration. By the three-way stopcock at the upper end of the collecting tube the first sample may be discarded, permitting the washing out of the tubing between the inner opening of *E* and the stopcock. A water manometer *M* is inserted between *E* and the collecting tube. As the mercury is allowed to fall at an even rate during the collection of a sample the water in the manometer rises during expiration to displace the mercury, as *E* is sealed off preventing the entry of air. At the beginning of inspiration when *E* is opened, air enters and the water drops to its previous level. Thus it acts as an indicator that *E* is being sealed off at the beginning of expiration and opened at the beginning of inspiration. Also by displacing the mercury when *E* is sealed off it greatly limits the negative pressure which would otherwise develop in the collecting system. In this manner the air sample may be collected at an even rate of speed over an indefinitely long period of time or during the period of two or three respirations depending on the rapidity with which the mercury is

allowed to fall. When it is desired to collect the sample rapidly it is necessary to stop the falling mercury during the expiratory phase to prevent the water of the manometer and the room air from being drawn into the collecting system.

The valve was made as small as possible in order to minimize the increase in dead space but sufficiently large to offer no resistance to breathing. It had approximately twice the dimensions shown in figure 1. With the tube *D* leading to the mask at least 1 cm. in diameter it was found suitable for all dogs weighing between 8 and 16 kgm. The dead space in the valve and in the mask connection amounted to approximately 13 cc. This was slightly increased by a small volume within the mask as the nostrils of the dog did not perfectly approximate the opening.

TABLE 1

DETAILS	SAMPLE NUMBER	SAMPLES COLLECTED FROM THREE EXPIRATIONS		SAMPLES COLLECTED OVER A PERIOD OF TWO MINUTES	
		Carbon dioxide	Oxygen	Carbon dioxide	Oxygen
		per cent	*per cent*	*per cent*	*per cent*
March 3, 1927, Dog II	1			5.20	13.93
Respiratory rate = 12 per minute	2	5.40	14.00		
Pulse rate = 76 per minute	3			5.20	14.30
	4	5.20	14.32		
	5			5.30	14.15
	6	5.30	14.33		
	7			5.40	14.20
Average		5.30	14.22	5.27	14.14
Average of 6 samples March 3, 1927, Dog III		5.08	14.49	5.10	14.50

The fact that the valve was not working on a narrow margin of expired alveolar air was shown by comparing alternate samples collected at different rates. Table 1 shows the results of two experiments in which samples collected from a small portion of each expiration are compared with samples collected from a relatively large portion of each expiration. The results are identical within the limits of normal variation.

To train the dogs to lie quietly throughout the course of an experiment was an unexpectedly simple matter. After two or three days' training they would lie on their sides wearing the mask and breathing quietly for one hour or even longer if necessary. Their quietness was shown by the fact that samples at 3 or 4 minute intervals over a period of one-half hour were of constant composition. At rest the dogs usually breathed at an even respiratory rate of 10 to 15 per minute, and this was not increased

by the wearing of the mask. It is presumed that the increased dead space was being compensated for by an increase in the tidal air but this was not sufficient to be noticeable However any excitement or anxiety was at once reflected in some hyperpnea resulting in a lowered carbon dioxide tension and raised oxygen tension of the alveolar air. In later experiments in which ventricular puncture became routine, although the dogs required no restraint and showed no change in breathing at the actual time of puncture some apparent anxiety was shown by a constant respiratory rate faster than usual. This phenomenon did not present itself in initial experiments, but after ventricular puncture had been carried out two or three times. The institution of puncture experiments reduced previous carbon dioxide tensions of 5.5 to 5.0 per cent to 5.0 to 4.5 per cent.

TABLE 2

1ST H.P. SAMPLE		2ND H.P. SAMPLE		AVERAGE H.P. SAMPLE		VALVE SAMPLE	
Carbon dioxide	Oxygen	Carbon dioxide	Oxygen	Carbon dioxide	Oxygen	Carbon dioxide	Oxygen
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4.70	15.58	5.35	14.45	5.02	15.01	5.60	14.90
5.25	14.65	5.48	14.40	5.36	14.52	5.48	14.84
5.20	15.22	5.54	14.75	5.37	14.98	5.60	14.45
5.27	15.11	5.58	15.10	6.42	15.10	5.42	15.23
5.08	16.14	5.12	16.08	5.10	16.11	5.28	16.09
4.90	15.88	5.03	16.27	4.96	16.08	5.38	15.57
5.48	15.74	5.63	15.38	5.55	15.56	5.48	15.93
4.68	16.25	5.25	15.35	4.96	15.80	5.35	15.30
5.07	15.57	5.37	15.22	5.22	15.39	5.45	15.30

It was considered of interest to compare the results of alveolar airs in man obtained by the valve with those obtained by the Haldane-Priestley method. Table 2 shows that the valve results agree quite closely with the second Haldane-Priestley sample and seem to be definitely higher in carbon dioxide tensions than the average of the two samples. This is in accordance with the results of Henderson (1927) on analyses of the alveolar air for ethyl iodide. He finds that the sample automatically collected from the last air expired approximates the second Haldane-Priestley sample. As the following experiments show a carbon dioxide equilibrium between arterial blood and air obtained by the valve there is also agreement with the results of Bock and Field (1924) on man in that the air which they found to be most nearly in equilibrium with the arterial blood was the second Haldane-Priestley sample.

COMPARISON OF THE CARBON DIOXIDE TENSIONS OF ARTERIAL BLOOD AND AIR OBTAINED BY THE VALVE METHOD. An analysis of the literature would

indicate that probably the carbon dioxide of the alveolar air is in equilibrium, or very nearly so, with that of the arterial blood. Bohr in 1890 compared the gas tensions of the arterial blood and "tracheal bifurcation air" of normal dogs. The air was collected from the tracheal bifurcation by means of a catheter and the blood gas tensions determined on hirudinised blood by the hemat-aerometer method. On the wide differences in tensions thus obtained he based the theory of gas secretion by the lungs. Krogh and Krogh (1910) working on rabbits and using an improved but somewhat similar technique came to the conclusion that the carbon dioxide tensions of alveolar air and arterial blood showed very little difference and were perhaps identical, the carbon dioxide tension of the blood averaging about 3 mm. above that of the air of the tracheal bifurcation. Experiments on the tortoise led them to similar conclusions. Peters, Barr and Rule (1921) presented results in man showing the carbon dioxide tension of the alveolar air obtained by the Haldane-Priestley method to be considerably lower than that of arterial blood (4 mm. to 11 mm.). Campbell, Hunt and Poulton (1923) in experiments on eight normal subjects found the carbon dioxide tension of the alveolar air in six cases definitely higher and in two cases lower than that of the arterial blood. Bock and Field (1924) reported a larger series of experiments on human subjects showing close agreement between the carbon dioxide tensions of alveolar air and arterial blood, that of the blood averaging 0.48 mm. higher than that of the air. They used as alveolar air the second Haldane-Priestley sample believing that the air so obtained most truly represents effectual alveolar air. Dautrebande (1926) reported a series of results in pathological cases in which the tensions showed even closer agreement than those of Bock and Field. The air used was alveolar air obtained by the usual Haldane-Priestley method. In our series of experiments an agreement is shown within the limits of experimental error.

The experiments were carried out on dogs at the end of a 15 to 20 hour fast. They were fed a constant diet at 4 p.m. daily. Gas analyses were made by the Henderson modification of the Haldane apparatus. Blood analyses were carried out in the constant volume apparatus described by Van Slyke and Neill (1924).

The dog was made to lie in a trough to obviate the necessity of changing the position of the animal for ventricular puncture. Over the puncture site, 1 per cent novocaine solution was injected intradermally and subcutaneously. When the dog had become accustomed to his position and was breathing quietly, synchronous samples of air and arterial blood were taken. The air was taken in the manner described above and the blood obtained by left ventricular puncture. Never during the experiments was it necessary to hold or restrain the dogs in any way. The ventricular puncture seemed to cause little or no discomfort. It is important to

emphasize that the collection of air was begun as the blood entered the syringe and was collected at such a rate that it terminated when the full sample of blood was obtained. As a rule it required about 45 seconds for drawing the usual amount of 15 cc. of blood.

For the collection of blood a 3.5 inch no. 18 needle on a 20 cc. syringe containing about 5 cc. of paraffin oil was used. The blood was immediately transferred under oil without contact with air into a tube containing potassium oxalate (200 mgm. per 100 cc. blood) and sodium fluoride (50 mgm. per 100 cc. blood). In this quantity potassium oxalate is well below the content shown by Peters, Barr and Rule (1921) to be capable of increasing the carbon dioxide combining power. Sodium fluoride in the above quantity has been shown by Evans (1922) to be sufficient to prevent glycolysis. The portion of the blood sample not used immediately was placed in a cold room.

A sample of blood was at once analysed for carbon dioxide and oxygen. The remainder of the blood was used for equilibrations with three gas mixtures in order to obtain three points on the carbon dioxide dissociation curve. While the sample of blood as drawn from the left ventricle was being analysed by one of us, two equilibrations were carried out by the other. The resulting samples were analysed for carbon dioxide and oxygen as soon as possible, one immediately, the other after standing for about 20 minutes. In the meantime the third equilibration had been completed and the resulting blood was analysed. As a rule all blood analyses had been completed 90 minutes after obtaining the blood.

The gas mixtures for equilibration were made up in a 10 liter spirometer. In each experiment the three mixtures contained approximately carbon dioxide tensions of 20 mm., 35 mm. and 50 mm., and oxygen tensions of 100 mm. Equilibrations were carried out by rotating in a water bath at 37°C. for 20 minutes, an apparatus devised by Prof. E. A. Doisy and represented in figure 2. It made possible the analyses of both blood and gas mixture following the equilibration, without involving temperature and pressure corrections. It consisted of two vessels, a large bottle, A, of 600 cc. volume and a tube, B, of 10 cc. volume, in free communication by tubes T_2 and T_3. The apparatus was washed out with the gas mixture for equilibration. 5 cc. of blood were pipetted into A through T_3 and the connections made as shown in the figure with B in free communication with A and tube T_1 clamped off. During the equilibration T_1 acted as a support for B to which it was attached by an elastic band. After five minutes of rotation T_1 was opened momentarily to the atmosphere to allow the gas mixture to reach atmospheric pressure. On completion of equilibration the apparatus was inverted while still in the bath, so that the blood passed from A into B by tube T_2. The clamps were closed on T_2 and T_3 between the two vessels and the latter were then disconnected. Oil was introduced

TABLE 3

	CORRESPONDING CURVE NUMBER	OXYGEN CONTENT OF ARTERIAL BLOOD	OXYGEN CONTENT OF BLOOD EQUILIBRATED AT OXYGEN TENSION OF ALVEOLAR AIR	DIFFERENCE IN OXYGEN CONTENT OF ARTERIAL AND EQUILIBRATED BLOODS	CARBON DIOXIDE TENSION OF ARTERIAL BLOOD	CARBON DIOXIDE TENSION OF AIR OBTAINED BY VALVE	DIFFERENCE
		vol. per cent	*vol. per cent*	*vol. per cent*	*mm.*	*mm.*	*mm.*
Dog II							
Apr. 6, a.m.		20.1	21.2	1.1	31.8	31.8	0.0
6, p.m.	1	19.1	22.0	1.9	28.6	26.6	+2.0
11, p.m.		21.0	22.6	1.6	34.4	34.4	0.0
13, a.m.	2	17.4	18.8	1.4	34.6	34.0	+0.6
13, p.m.		17.5	18.4	0.9	34.9	35.2	−0.3
18, a.m.	3	17.7	18.7	1.0	31.9	32.2	−0.3
18, p.m.	4	15.8	16.3	0.5	35.2	35.0	+0.2
21, a.m.	5	18.9	20.7	1.8	29.6	32.5	−2.9
21, p.m.	6	17.5	18.0	0.5	29.7	33.6	−3.9
Dog III							
Mar. 24, a.m.		17.0	19.8	2.8	33.6	33.6	0.0
24, p.m.	1	17.6	18.7	1.1	31.2	31.2	0.0
31, a.m.	2	16.4	18.1	1.7	32.5	31.8	+0.7
31, p.m.	3	16.2	17.2	1.0	30.9	30.9	0.0
Apr. 5, a.m.		18.1	19.3	1.2	32.1	32.0	+0.1
5, p.m.	4	17.5	18.9	1.4	21.3	28.4	−7.1
Dog V							
Mar. 30, a.m.	1	20.1	20.5	0.4	34.3	34.0	+0.3
30, p.m.		17.3	19.6	2.3	34.6	34.9	−0.3
Apr. 4, a.m.	2	17.3	19.8	2.5	28.6	28.6	0.0
4, p.m.		17.4	19.9	2.5	28.8	28.5	+0.3
9, a.m.	3	15.6	19.0	3.4	32.3	29.3	+3.0
9, p.m.		17.2	18.1	0.9	34.4	33.7	+0.7
14, a.m.	4	16.8	18.3	1.5	33.8	35.2	−1.4
14, p.m.	5	17.1	17.8	0.7	36.7	33.7	+3.0
Dog VI							
Apr. 1, a.m.	1	18.9	21.5	2.6	30.2	36.7	−6.5
1, p.m.		18.7	21.3	2.6	37.0	35.5	+1.5
7, a.m.	2	18.5	20.5	2.0	29.7	29.7	0.0
7, p.m.		17.6	19.5	1.9	30.9	27.6	+3.3
12, a.m.	3	17.8	18.4	0.6	33.5	33.6	−0.1
12, p.m.	4	16.7	18.8	2.1	35.1	35.1	0.0
16, a.m.	5	16.4	18.1	1.7	32.6	32.3	+0.3
16, p.m.	6	15.0	16.2	1.2	29.4	29.4	0.0
20, a.m.		14.4	16.4	2.0	28.7	28.7	0.0
20, p.m.	7	15.3	16.9	1.6	29.0	29.4	−0.4

into B through T_2 by means of a syringe, T_3, being opened at the same time so that the gas mixture was displaced outward. The blood was thus covered by oil while still at 37° and in contact with the gas mixture used in the equilibration. A sample of the gas in the bottle B was withdrawn through T_1 and analysed for oxygen and carbon dioxide, the results being the tensions at which the equilibration was carried out.

METHOD OF RECORDING RESULTS. From the results of the three analyses of the equilibrated bloods three points on the dissociation curve were plotted in the usual manner. The carbon dioxide tension of the arterial blood was then read on the curve so obtained. In table 3 a comparison of the carbon dioxide tensions of the arterial blood and the alveolar air has been prepared.

In spite of the fact that all three equilibrations were carried out with oxygen tensions approximately that of alveolar air (about 100 mm.) the variation in oxygen content was sometimes as high as one volume per cent although usually the differences varied from 0.2 to 0.5 volume per cent. Consequently in each case the carbon dioxide content of the blood, before being plotted, was corrected to the highest oxygen content obtained. The value of carbon dioxide content of the arterial blood as drawn was also corrected in the same manner so that it could be applied to the dissociation curve. For corrections the factor 0.34 derived by Peters, Barr and Rule (1921) was used. From the carbon dioxide content in volumes per cent is substracted the product of the factor 0.34 and the oxygen unsaturation in volumes per cent. It was originally the intention to equilibrate a sample of blood with air in each experiment to determine the oxygen capacity but after a few such experiments it was found that they yielded results no higher then the equilibrations at approximately 100 mm. oxygen tension. Consequently the highest oxygen content obtained in the three equilibrations was considered as the oxygen capacity of the blood.

The results of each experiment were plotted separately but it was found that in almost every case the results of two experiments performed on the same day (morning and afternoon) would lie on a common curve. Consequently in most cases a curve is the result of two experiments carried out on the same day.

ACCURACY OF RESULTS. The maximum error in the blood analyses with the constant volume Van Slyke apparatus was 0.2 volume per cent and the possible error in the analyses of gas mixtures was 0.1 mm. Due to the slope of the carbon dioxide dissociation curve 0.2 volumes per cent is approximately equivalent to 0.4 mm. Thus the total error expressed in carbon dioxide tension is 0.5 mm. In the figure expressing the difference between the carbon dioxide tensions of the alveolar air and arterial blood the maximum possible error is naturally doubled. Consequently all differ-

ences in table 3 not over 1.0 mm. tension may be considered to indicate equilibrium.

Discussion of results. The deviations from equilibrium between the arterial blood and the alveolar air are shown graphically in figure 7. Each difference is expressed to the nearest 0.5 mm. along the axis of abscissae and each dot represents one experiment. Considering the possible experimental error of 1 mm. 20 points are well within the zone of equilibrium, those plotted on the zero axis deviating less than 0.25 mm. The remaining 10 points show a random deviation, five of them showing a carbon dioxide tension in the air above that in the blood, which in the light of present knowledge is impossible. Consequently we feel justified

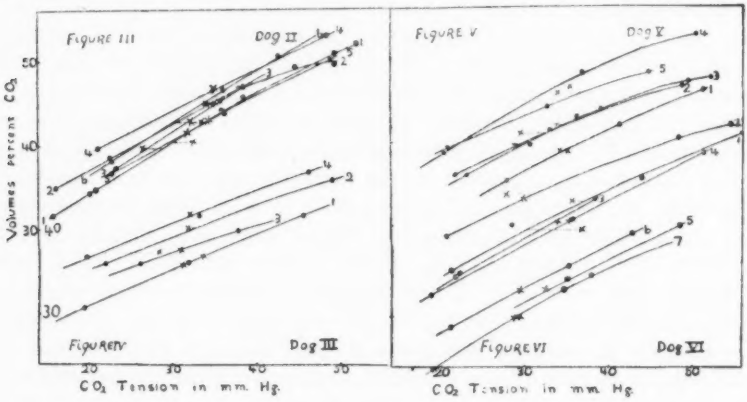


Fig. 3. In this and the following figures, ● = points derived on dissociation curve; x = carbon dioxide tension of alveolar air; ---------- = difference in carbon dioxide tension between alveolar air and arterial blood.

in concluding that the air obtained by the above method is in carbon dioxide equilibrium with the arterial blood.

In figures 3, 4, 5 and 6 the carbon dioxide dissociation curves between the limits of approximately 20 and 50 mm. tension are shown for the four dogs used in the experiments. The individual variation from day to day in the carbon dioxide combining power of the blood in the four series of results presented, varies from 2.5 volumes per cent in dog II to 8 volumes per cent in dog V. The greatest variation in all normal curves determined on the four dogs is little more than the greatest individual variation. The highest curve (dog V, April 14th) differs from the lowest curve (dog V, March 24th) by 9.8 volumes per cent at 35 mm. tension. In the case of dog VI the low curves shown for April 16th and April 20th are not con-

sidered normal. At this time the dog seemed listless and tired. The blood cells separated from the plasma more rapidly than usual and the plasma had a lipemic appearance. Also the oxygen capacity of the blood had fallen from a previous level of 21 volumes per cent to approximately 16 volumes per cent. The possibility of a change in the level of the curves due to an uncontrolled overventilation previous to the actual experiment, must be borne in mind. As a rule the curves determined during the morning and during the afternoon of the same day were identical. However such was not the case at all times. The widest variation during the day was encountered in dog III, March 31st, the afternoon curve lying approximately 2 volumes per cent below that of the morning. In the afternoon

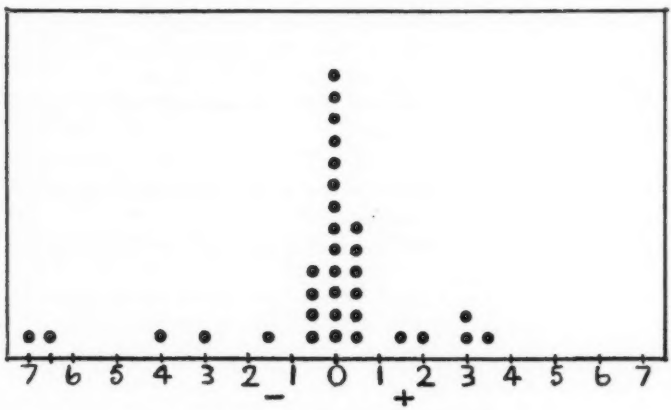


Fig. 7

the actual experiment happened to be delayed for about two hours. Throughout this period the dog was lying on the table breathing somewhat rapidly, the carbon dioxide tension of the alveolar air being rather low—30.9 mm. Henderson and Haggard (1918) have shown that the carbon dioxide combining power of the blood is lowered by a prolonged hyperpnea and such a possibility was considered in this instance. Moreover this might account for a certain degree of the day to day variation encountered.

In table 3 are compared for each experiment the oxygen content of the arterial blood with that of the blood equilibrated at an oxygen tension of approximately that of alveolar air. In no case does the arterial blood show an oxygen content as high as that of the equilibrated blood, the average being 1.6 volumes per cent lower. Assuming that the values for the equilibrated blood represent the oxygen capacity the average oxygen

saturation of the arterial blood is 91.7 per cent. This is somewhat below the figure of 95 per cent approximated in normal human subjects by various observers (Harrop, 1919; Stadie, 1919; Meakins and Davies, 1920).

SUMMARY

1. A method is described for obtaining alveolar air in normal unanesthetized dogs in which the sample consisting of the last air expired in a series of consecutive respirations is automatically collected.

2. When the method is applied to man the air collected approximates the second Haldane-Priestley sample.

3. Alveolar air collected by the above method was compared with respect to carbon dioxide and oxygen tension, with arterial blood drawn from the left ventricle synchronously with the taking of the air sample. By a series of such experiments equilibrium between the air and the blood was shown to exist for carbon dioxide but not for oxygen.

4. The variation of the carbon dioxide dissociation curve in the physiological region in dogs was indicated.

We wish to express our appreciation to Dr. Herbert S. Gasser for the instigation and direction of this research.

We are grateful to Dr. George H. Bishop and Dr. Ethel Ronzoni for many helpful suggestions.

BIBLIOGRAPHY

Bock and Field. 1924. Journ. Biol. Chem., lxii, 269.
Bohr. 1890. Skand. Arch. Physiol., ii, 236.
Dautrebande. 1926. Compt. Rend. d. Seances de la Soc. de Biol., xciv, 129.
Evans. 1922. Journ. Physiol., lvi, 146.
Harrop. 1919. Journ. Exper. Med., xxx, 241.
Henderson, Y. 1927. Journ. Physiol., lxii, 262.
Henderson, Y. and Haggard. 1918. Journ. Biol. Chem., xxxiii, 355.
1925. This Journal, lxxiii, 193.
Krogh and Krogh. 1910. Skand. Arch. Physiol., xxiii, 179, 200.
Krogh and Lindhard. 1914. Journ. Physiol., xlvii, 431.
Meakins and Davies. 1920. Journ. Path. and Bact., xxiii, 451.
Marshall. 1926. This Journal, lxxvii, 459.
Peters, Barr and Rule. 1921. Journ. Biol. Chem., xlv, 489.
Stadie. 1919. Journ. Exper. Med., xxx, 215.
Van Slyke and Neill. 1924. Journ. Biol. Chem., lxi, 523.

THE EFFECT OF INJECTION OF FOLLICULAR EXTRACT ON THE SEX ORGANS IN THE GUINEA PIG AND THE INTERACTION BETWEEN THE FOLLICULAR SUBSTANCES AND SUBSTANCES GIVEN OFF BY THE CORPUS LUTEUM[1]

LEO LOEB AND W. B. KOUNTZ

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Received for publication November 25, 1927

On the basis of our experimental analysis of the mechanism of the sexual cycle, we concluded that two separate phases must be distinguished in the sexual cycle of the guinea pig (1). The first one comprised the heat period and the period immediately following it. Experimentally we could show that the second, by far the longer phase, was essentially under the control of the corpus luteum and we designated it therefore as the lutein phase, in contradistinction to the first or follicular phase, so named by us because it coincided with the maturation of follicles and because there was some evidence that it was controlled by the maturing or the mature follicles (2), (3). However, we were aware of the fact that while, as far as the second phase was concerned, we had provided a direct experimental basis for the conclusion that it was controlled by the corpus luteum, the conclusion that the follicles controlled the first phase depended more or less on circumstantial evidence. Oestrus precedes the period in which the corpus luteum functions; it coincides with the maturation of ovarian follicles, and, as we pointed out, during maturation changes take place in the epithelium of the maturing follicles which suggest its active function. These observations made to us at least probable the significance of the follicular system in the regulation of the first phase. Subsequently Frank (4) and especially Allen and Doisy and their collaborators (5) showed that, through injection of the follicular substance, changes corresponding to the oestrus can be produced in certain rodents, and Allen showed furthermore that through such injections sexual maturity can be accelerated in young rats. These findings, which have been confirmed by a number of investigators, furnish apparently the direct experimental proof that the first phase is dominated by the maturing follicles; but still more recent

[1] A preliminary account of these investigations appeared in Proc. Soc. Exper. Biol. and Med., 1927, xxiv, 728.

investigations have shown that the effects of the follicular substance are by no means absolutely specific, inasmuch as several other substances act in a similar manner. Furthermore, one of us (Loeb) observed several years ago that the proliferation of the vaginal epithelium may begin in guinea pigs without the presence of mature or even of large follicles. We can therefore at best consider only that this function of the follicular extract in the sexual cycle is probable. However, certain additional conclusions of these investigators we consider erroneous, and we attribute the differences between the views which we expressed formerly and the more recently developed theories of the later authors in the main to two factors: 1. In the investigations of Allen, Frank and others the effect of the extract was usually determined solely by means of vaginal smears according to the method of Stockard and Papanicolaou (6). While this method is very convenient and provides a simple means of establishing the effectiveness of the injections, it does not give an insight into the actual changes which take place in the sex organs as the result of the administration of this substance. 2. There is an additional factor which renders the interpretation of their findings difficult, namely, the choice of the animals generally used. For the sake of convenience and economy, the large majority of these investigators, studying the effect of this substance, used in their experiments the smallest available animals, namely, the mouse and the rat. Now, as one of us pointed out previously, these two animals are peculiar in that their sexual cycle is very short, owing to the rudimentary character of their lutein phase. In consequence of this condition the follicular phase greatly predominates here, although under special conditions the lutein phase can become fully developed even in these two species. Owing to these two circumstances Frank, Allen and others arrived at the conclusion that there exists only one ovarian hormone dominating the sexual cycle, namely, the substance given off by the follicles and that the corpus luteum does not produce a substance active in the regulation of the sexual cycle but that it obtains whatever potency it seems to have from the fact that some of the follicular hormone is there deposited. According to this opinion the follicular substance contains therefore the only ovarian hormone. This conclusion seems to us at variance with the facts established by us previously, which proved the existence of independent functions in the corpus luteum.

In order to clear up, if possible, these discrepancies we decided to undertake an analysis of the effects of follicular extracts in the guinea pig, in which the mechanism of the sexual cycle has been experimentally analyzed by us and in which the cycle is more complete than in rat and mouse, both the follicular and the lutein phase being well developed. Moreover, instead of depending mainly upon the examination of vaginal smears, we made complete microscopical studies of the sex organs; in particular the

ovaries were cut into complete serial sections in almost all cases. In these investigations, which were begun almost two years ago, we used three different kinds of follicular extract, namely, one prepared by one of us (Kountz) and two extracts obtained respectively from Parke, Davis & Co., and from E. R. Squibb & Son. We designate these extracts as I, II and III respectively. I represents the strongest extract; II is of only moderate strength, and III approaches in strength but does not quite equal extract I. In the majority of experiments we used extract II, but we controlled the experiments with this material, whenever possible, by experiments in which we used the stronger extracts. In general, we arrived at the conclusion that while the use of relatively weak extracts may have certain disadvantages, it renders easier a finer differentiation of effects than the use of very strong preparations, and may thus have certain advantages over the latter, especially if we are able to control the results obained with the weaker extracts through additional experiments in which the stronger extracts are used. The injections were made as a rule subcutaneously, but in later experiments intraperitoneal injections were given in many cases.

The principal considerations underlying these investigations were then as follows: In former experiments we have shown that the cyclic corpus luteum, as well as the corpus luteum of pregnancy, prevents ovulation in the guinea pig (7), (1); furthermore as long as these corpora lutea function a typical heat does not occur, although the maturation of follicles is not interfered with during the ordinary cycle nor during pregnancy. On the other hand, as we have proven experimentally, it is possible to accelerate ovulation during the ordinary cycle through complete extirpation of the corpus luteum; similarly during early stages of pregnancy we succeeded in a number of cases in extirpating completely the corpora lutea without terminating the pregnancy; the latter continued for some time following the operation and through these means we could call forth experimentally an ovulation even during pregnancy. On the basis of these experiments we concluded that the corpus luteum is one of the principal factors regulating the duration of the sexual cycle. This conclusion was borne out by the fact that, at the time of a new ovulation, the corpora lutea functioning in the preceding cycle have begun to undergo degeneration.

There is a second function of the corpus luteum, namely, the sensitization of the uterine mucosa which makes possible the formation of placenta and placentomata. As stated in our earlier papers (8), (1) the corpus luteum begins to secrete a substance sensitizing the uterine mucosa very soon after its formation. The substance thus secreted gradually accumulates and the size of the placentomata, produced experimentally in the uterus, increases with the increasing amount of this substance given off, until the maximum is reached at about the sixth or seventh day following

ovulation. Subsequent to the eighth day the secretion suddenly ceases or at least it becomes ineffective. Some sensitization has already occurred in the course of the fourth and fifth day or even somewhat earlier. Extirpation of the corpora lutea in the early period of the sexual cycle, especially if carried out six days following ovulation, does not therefore necessarily prevent the formation of placentomata, although such structures formed under these conditions are usually small. It follows from these observations that we may expect that under the influence of a fertilized ovum, which has been embedded in the uterine mucosa following fertilization, a certain amount of maternal placenta may be produced, even if the extirpation of the corpora lutea has taken place at the time of nidation or even at a slightly earlier period. The contradiction which Drummond and Robinson and Asdell (10) see between our conclusion that a substance given off by the corpus luteum is necessary for the production of the placenta and our statement that pregnancy may develop and persist for some time in cases in which the corpora lutea have been completely extirpated later than three days following ovulation does therefore not exist. Furthermore, our observations in a case of experimentally produced extrauterine pregnancy in the guinea pig prove that the fertilized ovum can become embedded in the host tissue and undergo at least the first stages of development even without the formation of maternal placenta, which in our experiment was entirely lacking (13). We may therefore conclude that the amount of decidua needed in the early stages of pregnancy is very small. Our experiments have thus shown that it is not the pregnancy as such which prevents ovulation, but the function of the corpus luteum. Of course it is to be expected that in a number of cases operative interference with the generative organs in the pregnant guinea pig will lead to a termination of pregnancy. Furthermore it may be expected that different species of animals show a different degree of sensitiveness to ovarian operations during pregnancy and it is therefore not surprising that Parkes and Bellerby (11) find that extirpation of the corpora lutea in the mouse produces a premature interruption of pregnancy.

While, as stated above, in the pregnant guinea pig the resistance to a new ovulation can be removed through extirpation of the corpora lutea, we found that accompanying and following ovulation the uterine mucosa is rigid in the pregnant animal and does not undergo the changes characteristic of the sexual cycle; we must therefore assume that this latter inhibition is independent of the function of the corpora lutea but is produced through some other factor connected with pregnancy (1). Since then the sexual cycle can be separated into two phases and the corpus luteum has at least two functions, then these functions must in some way interfere in the effects of the injection of follicular extract, and we wished accordingly in our present investigations to determine in the first place, whether the functioning

corpus luteum tended to render more difficult the ovulation which according to expectations should be caused by the injection of this extract. However, to our surprise we found in the course of our investigations that the injections of the follicular extract, instead of calling forth ovulation in the guinea pig, on the contrary prevented it and thus led to a sterile condition of the animal during the time that the injections were effective. In accordance with our earlier experiments we found that the large, persistent corpus luteum of hysterectomized and pregnant guinea pigs tends definitely to counteract certain heat changes which otherwise would have been caused by the administration of follicular extract. On the other hand, the ordinary cyclic corpus luteum, which likewise prevents ovulation, was not able to prevent the heat changes; whether it is able to oppose and weaken the latter, needs still further investigation.

As to the second function of the corpus luteum, which we established, namely, that of sensitizing the uterine mucosa in such a way that mechanical stimuli subsequently applied lead to the production of placentomata (9), it was of interest to determine whether the follicular extract can take the place of the corpus luteum in this respect. Such an effect should be expected if the conclusions of those authors which consider the corpus luteum merely as the depository of the follicular substance were correct. Our experiments not only prove that this is not the case but that on the contrary the injection of this extract tends to prevent the predecidual changes in the uterus which normally occur five to seven days following ovulation under the influence of the corpus luteum.

There is a third function of the corpus luteum, namely, that of producing proliferation of the mammary gland (12). However, this function it shares with the factors which dominate the follicular phase, in all probability mature or maturing follicles. Under these conditions it was therefore to be expected that the corpus luteum would not tend to inhibit the proliferation of the mammary gland which takes place under the influence of the follicular extract. This anticipation was confirmed in our experiments.

In order to answer these questions we undertook a series of experiments in which we injected follicular extracts in various kinds of guinea pigs.

A: *Injection of follicular extract in sexually immature guinea pigs.* Six sexually immature animals in this series received injections of follicular extract and four immature guinea pigs which were not injected served as controls. Our experiments lead to the conclusion that injections of follicular extract in immature guinea pigs accelerate the maturation of ovarian follicles; but instead of rupturing, the mature follicles thus produced retrogress and undergo typical atretic changes. It was not possible through these injections to induce an ovulation and this was true of animals which were injected with extract of only moderate potency, as well as of a guinea pig injected with the more potent extract I. The extract caused a definite

proliferation of the vagina which was more marked in the case in which the more potent extract I was used. It is of interest that the proliferation was most pronounced and the keratin formation most common in the proximal part of the vagina. In all cases the mammary gland showed early stages of proliferation. In contrast to the ovaries, vagina and mammary gland, the uterus in this series of six animals did not show microscopically any proliferative changes except in two animals. In addition in two animals of series A incisions had been made in the uterus at a certain time during the period of injection. In these guinea pigs noticeable proliferative changes in the uterus were lacking. Out of seven immature guinea pigs injected with extract, two animals showed a slight proliferation of the uterine mucosa. There was one animal in series B, likewise injected with extract II at approximately the same time that the guinea pigs of series A were injected, which showed likewise a slight proliferation of the uterine mucosa. These changes in the uterine mucosa represent a marked oestral change. As we have shown previously, at this phase of the cycle the neck of the glands develops a high cylindrical epithelium, in which the epithelial cells multiply by mitoses, while the connective tissue cells and nuclei of the mucosa undergo enlargement and may show likewise some mitoses. In these two cases, as well as in the case of series B, these heat changes were quite pronounced. It is of interest that they were obtained by the injection of extract II which is of only moderate strength and that the three animals in which these relatively marked effects were observed in the uterine mucosa were injected at about the same time and therefore presumably with the same extract.

B: *Injection of follicular extract in underfed guinea pigs.* Six guinea pigs with an initial weight varying between 300 and 335 grams were underfed for several, usually three, days and then injected with follicular extract; or, in some cases, injections were begun simultaneously with the underfeeding or one day later. The guinea pigs were injected daily with 1 cc. of follicular extract II over a period varying between seven and fourteen days, during which the underfeeding continued. The animals were either immature at the time when the experiments were begun, or they had entered the period of maturity only a short time previously.

As to the ovaries of the injected animals, our findings may be summarized as follows: In accordance with our earlier experiments we find that in the control animals the underfeeding produced on the whole a hypotypical condition of the ovaries, in which the follicles develop only to a certain size and then begin to degenerate, and this effect was especially marked in the immature guinea pigs. A new ovulation was impossible in accordance with the lack of mature follicles. In the injected guinea pigs, on the other hand, the follicles grew to a larger size and in several animals the follicles matured, although new ovulations did not occur. However, the injec-

tions did not in every case prevent a hypotypical condition of the ovaries, especially in the immature animals. In none of the injected guinea pigs was the life of the corpus luteum prolonged through the injections, although the growth of the follicles was promoted, notwithstanding the deficient amount of food which was available. The ovaries on the whole were somewhat larger and they contained larger follicles in the injected animals than in the controls. The vagina in the injected guinea pigs opened between the sixth and eighth day after the beginning of the injections, except in two animals in which an opening of the vagina was not observed. In the latter two guinea pigs mature follicles did not develop; and in one of these animals the follicles became atretic before they had reached large size. There is thus a correspondence between the effect of the extract on the condition of the ovaries and on the opening of the vagina. In general, we notice in this series a correspondence between the effects of the injections on the ovary and on the vagina. In those cases in which we found in the ovaries mature or almost mature follicles, the proliferation of the vagina had progressed farthest and keratinization had begun. In those cases, on the other hand, in which the guinea pigs were immature and the effect of the injections did not lead to the production of maturing follicles, the effect on the vagina was least pronounced; it was lacking or almost lacking in guinea pig 6, an immature animal in which the underfeeding caused a decided hypotypical condition of the ovaries and in which the injections extended over a period of seven days. However, the vaginal epithelium may proliferate even in cases in which the follicles do not develop fully and the ovaries show a hypotypical condition of moderate intensity. In such animals we have to deal with a direct action of the follicular extract on the vagina and not merely with an indirect one exerted through the ovaries. The condition of the uterus corresponded to the effects of underfeeding previously observed by us (14).

The influence of the injections extended also to the uterus. It caused here, in general, a better circulation, in some cases visible to the naked eye as a hyperemic condition; it also prevented in some animals the shrinking of the epithelium which we generally find in the controls. In one case we found a marked activity in the connective tissue and glands characteristic of heat. In the animal in which the ovaries were definitely hypotypical the uterus was quiescent and atrophic.

There is again noticeable a certain correspondence between the effects of the follicular extract on the uterus and on the ovaries, although it is difficult at present to determine how far the effect of the injections on the uterus is a direct or an indirect one, exerted through the ovaries. However, on the whole, the effects observed in the uterus are small as compared to those which we found in the vagina.

The mammary glands in the injected animals were in all cases proliferat-

ing, except in guinea pig 6, in which in general no or only a very slight effect of the injections was observed. In the controls, the mammary glands consisted usually only of small ducts lined with low epithelium in which mitotic activity was lacking. The follicular extract thus produced a stimulating effect on the mammary gland. As in the case of the vagina this effect is at least partly a direct and not an indirect one exerted through the ovaries.

We see then that to some extent the follicular extract can overcome the effects of the underfeeding on the generative organs and can direct to them sufficient blood supply and nourishment to prevent atrophy; and it may even cause certain proliferative changes. But again, as in the preceding series, the injections were in no case able to induce an ovulation.

C: *Injection of follicular extract in castrated guinea pigs.* In standardizing the strength of the follicular extract we tested its effects on castrated guinea pigs; we determined the length of time and the number of injections necessary to produce an opening of the vagina. Furthermore, we examined the sex organs of five of these animals after they had received a series of injections. In these five cases the length of time between castration and the beginning of injections varied between four weeks and four months. In three guinea pigs injections of extract II were given twice a day; in a fourth animal this extract was injected three times a day, and in the fifth case extract I was injected twice daily. The weight of these animals varied approximately between 600 and 900 grams. The vagina opened in these cases usually five to six days after the beginning of the injections. If the examination occurred several days after the opening, the injections were continued and the vagina remained wide open during these additional days. In all cases the injections caused a proliferation of the vagina, the lining of which became thus transformed into squamous epithelium. The latter was covered either by the raised up cylindrical epithelium or by a layer of keratin into which the upper part of the squamous epithelium had been changed. However, keratin was not found in all cases. When present at all it developed first in the proximal portions of the vagina, where also the squamous epithelium was generally thickest; it was usually lacking in the distal portion, where the epithelial proliferation in general was less pronounced. Keratin was best developed in the case in which extract I was used; in this animal in which the examination took place eight days after the beginning of the injection, the keratin was found cast off in the cavity of the vagina in the form of lamellae and in addition there were found in this cavity desquamated, round, keratinizing cells. Moreover, some epithelial pearls had developed in the thick layer of stratified epithelium but no mitoses were visible in this case. As usual the fibrous tissue underneath was dense. In the other cases in which the proliferation and subsequent differentiation had not yet progressed so far, mitoses were usually found in the lowest layer of the squamous epithelium. Among this

group of additional animals a beginning of keratinization was observed only in one case in which the injections were continued longest, namely, during a period of five days following the opening of the vagina which occurred six days after the beginning of the injections. The number of polymorphonuclear leucocytes migrating through the vagina was usually small. The transition between the cervix in which no marked proliferation took place and the proliferating vagina was gradual. The cervix was usually papillary and covered by one layer of cylindrical epithelium underneath which there was visible in some places a layer of low cuboidal epithelial cells. The uterine surface epithelium was in some cases cylindrical, in others cuboidal; there were usually some mitoses found in this layer. The epithelium of the glands was low and without mitotic activity. The connective tissue was fibrillar and its nuclei were small. There was no proliferation noticeable. Here and there some hyperemic and edematous areas were found in the mucosa, and the connective tissue assumed in such places sometimes a myxoid character; but this condition was not everywhere present and it was lacking in some cases almost altogether. In one animal a hemorrhage had occurred in one area of the uterine wall. A predeciduomatous condition of the mucosa was not observed even in the case in which the most active extract had been used and which had caused a very marked proliferation in the vagina.

The mammary gland was in all cases proliferating; mitoses were frequent especially in the epithelium, but in one case they were observed also in the stroma. The number of acini which had developed varied in different cases. Lymphocytes and some eosinophiles were found in the connective tissue.

The changes in the castrated animals were produced through the follicular extract directly without the coöperation of substances which were given off by the ovarian follicles. These effects were very definite, as far as vagina and mammary gland were concerned; on the other hand they were very slight or lacking altogether in the uterus.

D: *The effect of injections of follicular extract, which were begun on the first or second day following oestrus.* In a series of experiments we studied the effect of follicular extract in guinea pigs injected at various periods of the normal sexual cycle. We thus wished to determine the interaction between those factors which vary during different phases of the cycle and the effects of the extract. First we shall consider the effect on guinea pigs in which the injections were begun on the first and second day following the period of heat.

In two guinea pigs the injections were begun during the period of heat or soon afterwards and continued for six days. The sex organs were removed for examination six or seven days following heat. In these experiments we wished to determine the effect of the injections of the follicular

extract on the predecidual proliferation of the uterine mucosa which normally occurs five to seven days following heat; in particular was it of interest to determine whether these predecidual changes would be intensified through these injections. In the first guinea pig weighing 530 grams, the injections with extract III were begun one day after heat and continued twice a day for six days. One cubic centimeter was injected each time. Examination took place eight days after heat, on the day following the last injection. The ovaries showed a condition corresponding to this stage of the cycle. Medium sized to large growing follicles were observed. The corpus luteum was well preserved and showed loose connective tissue in the center. Follicles in the last stage of connective tissue atresia and some retrogressing yellow bodies were also seen. The vagina, which was fully open six days after the beginning of the injections, was lined with squamous epithelium in which mitotic proliferation was noticeable and which was covered by a layer of raised up cylindrical epithelium; the squamous epithelium was thicker in the proximal than in the distal portion of the vagina. Some keratinization had occurred in places. The epithelium of the cervix itself consisted of a layer of cylindrical cells. In the mammary glands there was active mitotic proliferation of the ducts. Some mitoses were also seen in the connective tissue. Of particular interest in this experiment was the condition in the uterine mucosa. Here we found that instead of increasing the predecidual proliferation of the mucosa the injections of follicular extract prevented it. The connective tissue was finely fibrillar; there was distinct hyperemia present and the capillaries pressed in places on the epithelium, which latter was in a resting state. The glands did not show any mitotic proliferation in contradistinction to the high cylindrical surface epithelium. These findings differ very markedly from those which are seen in normal animals at this stage of the cycle.

In an animal in which the injection began two days after oestrus, the vagina opened three days after the beginning of injections with extract II. The examination took place six days after heat. The condition found in the ovaries corresponded approximately to the period of the cycle as far as the character of the follicles and of the corpus luteum was concerned. The vagina showed mitotic proliferation, the squamous epithelium raising up the cylindrical epithelium, but no keratin formation had as yet taken place. The uterine mucosa was hyperemic, but fibrillar in character. Some hemorrhages and edematous fluid raised up the uterine epithelium in certain places. The glands were small and without mitoses. No predecidual changes had taken place but as usual the mammary gland was proliferating. Again in this case the injections prevented the predecidual proliferation of the uterine mucosa.

From these experiments we may conclude that in these cases in which injections were begun at an early period of the cycle, the vagina opened at an

early date and underwent or continued to undergo proliferative changes which did not lead to the formation of keratin. On the other hand, proliferation in the uterine mucosa was not only not intensified, but on the contrary it was apparently prevented through the injections. As usual proliferation was caused in the mammary gland through the injections of follicular extract.

E: *Effect of injections of follicular extract which were begun three and four days following heat.* In four experiments the injections with extract II were begun three days and in two experiments four days following heat and continued for seven consecutive days; the examination took place nine or ten days following heat, except in one guinea pig which received injections only during a period of five days and was examined seven days after oestrus. In this whole series the effects of the injections were very slight as far as proliferative changes in the vagina are concerned; only in one case a slight proliferation was observed. The uterus was usually hyperemic, without showing any signs of predeciduomatous proliferation. In the mammary gland some proliferation was usually noticeable. In the ovaries mature follicles were lacking in accordance with the period at which examination took place. This lack of marked effects is at least in part due to the fact that injections continued over a period of only six and seven days and that extract II used at that time was weaker than it was in subsequent experiments. In addition it is possible that the period of the cycle at which the injections were made influenced the results.

F: *Experiments in which the injections of follicular extract were begun five, six and seven days following heat.* Three experiments were made in this series. In all cases extract II was injected twice daily during a period of six days. In none of the animals thus injected was there a full opening of the vagina at the end of this period. While the proliferation of the vagina was relatively slight, partly owing to the use of extract II, which was especially weak at this time, and partly due to the fact that the injections extended only over a period of six days, there was observed in these cases a somewhat more energetic proliferation than in former series in which the injections were begun at a slightly earlier period of the cycle. The ovaries showed the typical picture of a combination of well preserved follicles and of follicles in various stages of atresia, the later stage of development of follicles in this as compared to the preceding series being in accordance with the later period of the cycle at which the examination took place, and there were mature or nearly mature follicles present in all of these guinea pigs. Hyperemia of the uterus was noticeable, but predecidual proliferation was lacking in all cases. As was generally the case, after injection of follicular extract, ovulation did not occur during this period of injection.

G: *Experiments in which injections were begun ten and twelve days following heat.* An ovulation had not occurred in these cases. The intensity

of proliferation of the vaginal epithelium depended at least in part upon the strength of the extract used. The injections, although causing congestion of the uterine mucosa, did not produce a predecidual proliferation. The mammary gland was proliferating in all cases.

H: *Injections of follicular extract extending over a period of two and a half to four weeks.* In five experiments the injections extended over a period varying between twenty-one and twenty-seven days and in an additional case they extended over a period of seventeen days. The injections were begun either on the day of heat or within the next five days following oestrus. In these experiments we wished to determine whether even in the case of long continued injections of follicular extract ovulation could be prevented during the period of the experiment; and in addition whether a cumulative effect on the sex organs would become noticeable under such conditions. In these animals following the first opening, the vagina regularly closed and opened during periods varying usually between two and five days. As far as the effect of the injections on ovulation is concerned, these experiments confirm our general conclusion that the injections inhibit ovulation. None of the guinea pigs that received two injections daily ovulated during the period of the experiment. Only one animal which received a smaller quantity of the extract, namely, one daily injection of preparation II, ovulated in all probability at the time of the first opening of the vagina following the beginning of the injections approximately thirteen days preceding examination and fourteen days following the preceding heat. This animal is the only one in all our experiments in which an ovulation was observed. The uterus was usually found to be hyperemic and its cavity filled with fluid at the time of autopsy. The mucosa was found to be in a resting state, yet there was usually some congestion of the vessels. The vagina was definitely proliferating. Mitoses were present and usually numerous in the squamous epithelium; occasionally also some amitoses were seen.

The long continued injection of follicular extract of moderate strength produced a more marked proliferation of the vagina than the injection extending over a short period; however, the proliferation remained rather limited and a markedly cumulative effect was not noticeable. The effect consisted essentially in prolonging the period during which proliferation took place rather than in intensifying the effect of the injections, although a slight intensification has apparently taken place. The mammary gland was usually actively proliferating and showed in all cases many mitoses. The acini were generally lined with a high epithelium. However, in case e, in which the animal was very young, only proliferating ducts were seen. In the stroma some eosinophiles and lymphocytes were found.

I: *Experiments in which in the course of the injections of follicular extract incisions were made into the uterine wall.* In former experiments one of

us has established the fact that after the uterine mucosa has been sensitized by the corpus luteum, mechanical stimulation of this tissue will lead to the production of placentomata. Thus it was made possible to produce experimentally at any point of the uterine mucosa and in any desired number maternal placentas identical with those which are normally produced in restricted areas through the stimulation of the mucosa by the fertilized ovum. It was of interest to determine whether continued injections of follicular extract likewise sensitized the uterine mucosa, so that incision made into the wall of the uterus after a number of subcutaneous injections had been given would lead to the production of placentomata. We chose for this series immature guinea pigs because two of the three injected guinea pigs, in which definite proliferations in the mucosa of the uterus indicating oestral changes were seen, had been immature at the time of the beginning of the injections; in one additional case we had to deal also with a young guinea pig which had been underfed.

From these experiments we may conclude that in contrast to the action of the extract on vagina and mammary glands (which were both in a state of proliferation) the extract is without any marked effect as far as the uterus is concerned, and furthermore we observed that mechanical stimuli which otherwise, when acting in combination with substances given off by the corpus luteum have very far reaching effects on the uterus of guinea pigs, are without any effect if in the absence of the substance given off by the corpus luteum the animal receives a series of injections of follicular extract.

J: *Injection of follicular extract in hysterectomized guinea pigs.* In former investigations we have shown that in the guinea pig hysterectomy prolongs markedly the life of the corpus luteum which latter, under these experimental conditions, reaches the size and structural characteristics of the corpus luteum of pregnancy (15). Both kinds of corpora lutea act similarly in preventing ovulation and thus interrupting the normal sequences in the sexual cycle and in addition they cause similar changes in the various sex organs. In particular do they cause a complete cessation of growth processes in the epithelium of the vagina, notwithstanding the continued maturation of follicles following extirpation of the uterus; but instead of rupturing they undergo atresia under those conditions. In both cases the mammary gland proliferates markedly and becomes transformed into a large glandular organ even if previous to the hysterectomy it consisted merely of a system of ducts. The condition thus created through hysterectomy closely resembles the state in the sex system produced in pregnancy. These findings suggested the following experiments in which we injected follicular extract into guinea pigs previously hysterectomized. Altogether seven experiments of this character were carried out.

At periods varying between three to five days following heat, the uterus

was almost completely extirpated, except that usually a small remnant was left near the cervix. The time following the hysterectomy when the injections were begun differed in different cases.

Our results in this series showed a lack of proliferation in the epithelium of the vagina, indicating thus that injections of the follicular extract are not able to overcome the effects of the persistent enlarged corpora lutea in these animals. There was also an increased resistance to the opening of the vagina, due apparently to the same cause, though a strong extract could overcome its action. The cessation of ovulation also observed involves two factors, *1*, the injection of the extract, and *2*, the persistence of the corpora lutea. The mammary gland was found markedly proliferated, but not more so than occurs in hysterectomized and non-injected animals.

K: *Injection of follicular extract in pregnant guinea pigs.* We have previously shown that the effects of hysterectomy and of pregnancy on the sex organs and sexual cycle in the guinea pig are very similar. It was therefore of interest to compare the effect of injections of follicular extract on pregnant guinea pigs with those which we had previously observed in hysterectomized animals. Three experiments of this kind were made; the stage of pregnancy in these guinea pigs differed somewhat in different cases. In no case did the injections cause an opening of the vagina. The state of the ovaries resembled closely that found in hysterectomized guinea pigs; especially the corpora lutea were essentially of the same character in both cases. The injections evidently did not produce any noticeable change in the condition of the ovaries. As was to be expected, ovulation did not take place in these animals, but instead the mature follicles underwent atresia. There was no sign of any proliferation in the vagina, the condition of which, especially in the somewhat further advanced stages of pregnancy, was identical with that seen in hysterectomized guinea pigs; in both kinds of animals the injections of the extract were quite ineffective to this organ. The mammary gland, in which acini had been produced, was in all cases proliferating, and mitoses were occasionally seen also in the stroma.

In one case we examined, in addition to the organs of the pregnant guinea pig, vagina and mammary gland of the embryos. The epithelium of the vagina consisted of one layer of cylindrical epithelium; proliferative changes were lacking. In the mammary gland we found a system of ducts with a few mitoses. There were not noticeable any special proliferative changes which might have been caused by the injections of the follicular extract in the organs of the embryo. We may therefore conclude that similar to the condition found in the hysterectomized guinea pigs, the corpus luteum prevents proliferation of the vagina and inhibits its opening also in pregnant animals. The inhibiting effect on the opening of the vagina is less pronounced and is therefore especially noticeable if we use extract of only

moderate strength. It is very probable that if we had used extracts I or III instead of extract II, we might have obtained an opening without a proliferation of the vagina.

In recent experiments Parkes and Bellerby (11) observed that injections of follicular extract in the pregnant mouse tend to lead to a termination of pregnancy. They found that large amounts of extract are required in order to obtain this result in later stages of pregnancy than in earlier stages. However, there is no reason for assuming on the basis of these experiments that only the corpora lutea of pregnancy exert an inhibiting effect on ovulation and oestrus, and that cyclic corpora lutea are not able to exert such an effect. On the contrary, such an inhibiting effect also of the cyclic corpus luteum has been shown by one of us in our earlier experiments (7) and confirmed more recently by Papanicolaou and Stockard (16). In this connection it may be stated that M. G. Smith (Johns Hopkins Hosp. Bull., 1926, xxxix, 203) observed an interruption of pregnancy in the rat as a result of injections of follicular extract.

Discussion and conclusions. 1. In the majority of our experiments we used a follicular extract of only moderate strength (extract II); but in some cases we controlled these effects by the use of stronger extracts (extracts I and III). While apparently the use of a less active substance is disadvantageous, because under certain conditions we may thus obtain negative results, when by the use of stronger ones we might have obtained positive results, it has on the other hand also some advantages, inasmuch as it makes possible a finer shading of results and enables us to discern differences where they might have been obscured by the administration of active substances producing maximum effects. However, in future experiments it will be necessary to carry further the comparison between the action of very potent and of less potent extracts in order to make our analysis more complete.

2. As to the effect of the extract on the ovaries, it accelerates the maturation of follicles in immature guinea pigs; it is furthermore able to moderate the tendency to the production of hypotypical ovaries which results from undernourishment or even to prevent the development of the hypotypical state. Administration of the extract seems to call forth a condition which enables previously undernourished ovaries to attract foodstuffs to themselves which would otherwise have been used for different purposes in the animal; it deflects such substances to the generative organs. However, in our experiments we were unable so far to counteract entirely the effects of undernourishment; our success in this respect was only partial. It is possible that by the use of more potent material, we shall be able to obtain a more marked influence, although so far there does not seem to be in our experiments a definite superiority of the stronger extracts over the weaker ones, as far as their effects on the follicles are concerned. While there is

noticeable in these two series of experiments a distinct influence of the extracts on the maturation of follicles, such effects were not observed in the other series of experiments which we carried out; in particular we did not find a more rapid maturation of follicles in experiments in which we used the extract during the period of the sexual cycle; nor did there seem to be an increase in the number of mature follicles in pregnant or hysterectomized guinea pigs. As far as the corpus luteum is concerned, the injections of the extract did not cause a prolongation of the life of this organ; retrogressive changes set in at the usual time and in some cases even at a very early period of the sexual cycle. On the other hand, the extract did not lead to a shortening of the life and function of the corpus luteum in pregnant or in hysterectomized animals. Definite effects on the corpus luteum seem therefore to be lacking or at best they must be very slight.

There is however one very striking effect of the injections on the follicles which we observed in every case in which two injections of the extract were given daily. Ovulation was suspended in every guinea pig throughout the period of injections. In this respect the extract acts apparently like the substance given off by the corpus luteum which, as our previous experiments have shown, prevents the rupture of follicles as long as it is intact. However, there is a discrepancy between this effect, which follows the intermittent administration of follicular extract, and the effect of follicles functioning under normal conditions during the sexual cycle, where apparently the follicles give off a hormone which acts in a similar way to the extract on the vagina but without preventing ovulation. This effect of the injections must be due either to the abnormal mode of administration which only partly imitates conditions as they take place in the normal guinea pig, or they represent non-specific effects of the follicular substance, or the substances injected experimentally differ in some respects from those acting on the normal animal. In this connection it is of interest to refer to the recent experiments of L. Haberlandt who observed that following transplantation of ovaries or administration of corpus luteum extract or placenta, copulation in rabbits did not lead to pregnancy.

There is another discrepancy between the action of follicular substance under normal conditions and under the conditions of our experiments. In immature and undernourished guinea pigs we notice an accelerating effect of the extract on the maturation of follicles. If this effect were always present, the follicles which prepare and give off the active substance should accelerate the growth and maturation of other, smaller follicles, and the greater the number of active follicles the more rapid should be the maturation of the remaining follicles. An action, not unlike an autokatalytic process, on the part of the growing follicles, would thus prevail. Such a progressive rapidity in the development of follicles is not observed. Even the injection of the extract during the normal cycle does not apparently

produce a noticeable acceleration in growth of the follicles. There is of course the possibility that such an effect is obscured through other counteracting factors which automatically limit the cell proliferation in the granulosa in such a way that a definite proportion between the different kinds of follicles is maintained. There is also the possibility that the corpus luteum may regulate this effect. On the other hand, we have good reason for assuming that the ovum gives off a substance which causes the continued growth of the follicles (17).

3. The effect of the injections on the proliferation of the epithelium of the vagina is quite definite. Under the influence of the substances injected, there takes place an active mitotic cell multiplication which increases considerably the thickness of the squamous epithelium and may lead to the production of keratin. However, the readiness with which these proliferative processes are induced, differs very much under different conditions and in the different series of our experiments. While the effect is quite definite in the majority of our series, it is lacking altogether in the hysterectomized and in the pregnant guinea pigs. In these latter animals we find in general one layer of cylindrical epithelium lining the vagina, although there may be in places a second layer of flat cells underneath it. We were not able by means of injections of follicular extract to cause a transformation of this cylindrical epithelium into squamous epithelium. The reason lies in the action of the one factor that these types of guinea pigs have in common, namely, a persistent corpus luteum of relatively large size. The effects of this persistent large corpus luteum on the vagina cannot be overcome in such animals through the injection of extract, in contradistinction to the effects of the ordinary cyclic corpus luteum which latter is not able to prevent vaginal proliferation. In a similar way, in animals which have a persistent, large corpus luteum, the opening of the vagina under the influence of the follicular extract is made more difficult than in animals in which merely a cyclic corpus luteum is functioning, or in immature and castrated animals in which corpora lutea are lacking altogether. While this inhibiting effect on the opening of the vagina can be overcome by the use of very potent extract, we have so far not been able to overcome by these means the inhibiting effect of the corpus luteum on the proliferation of the vagina. As far as the difference between the cyclic and persistent corpora lutea is concerned, at the present time we are not yet justified in concluding that the action of the persistent corpus differs from that of the cyclic corpus luteum in a qualitative and not merely in a quantitative manner. We know that also the cyclic corpus luteum inhibits ovulation and with it the accompanying heat. On the other hand, there is the possibility that the persistent corpus luteum does not only produce a larger quantity of the inhibiting substance, but produces a different and more effective kind of active substance. There is an indication that the cyclic corpus luteum not

only prevents ovulation but in addition exerts a distinctly, although only weakly, inhibiting effect on the proliferation of the vagina. The fact that the proliferation in the vagina was very weak in the animals injected during the cycle, and especially in those guinea pigs in which the injection began three to four days following oestrus, suggests such a conclusion. However, it is possible that the mildness of this effect was due not to the corpus luteum, but to the use of a relatively inactive extract and to the relatively short period of time during which the injections were given. Further experiments must decide this question.

The action of the extracts on the proliferation in the vagina can to some extent be intensified by prolongation of the period during which the injections are given, but more effectively still by the use of more potent extracts. It is especially through the effect of the various extracts on the proliferation of the epithelium of the vagina that the different extracts can be graded as to their potency. The action of follicular extract on the vagina is a direct and not merely an indirect one, acting merely through the ovaries. This is proven by the marked effect of its use in castrated guinea pigs. This is especially significant in view of the considerable average weight of this latter type of animal, which reduces the relative amount of the substance given per unit of weight. An effect on the vagina can also be obtained in underfed guinea pigs in which the ovaries have become moderately hypotypical. However, in those cases in which the influence of underfeeding on the ovaries was most pronounced, and in which the injections were least able to counteract it, they were likewise least effective as far as their influence on the vagina was concerned.

While by the use of more potent extract we were able to intensify the effect of the extract on the proliferation of the vagina, we did not succeed in obtaining this result by giving three instead of two daily injections of a weaker extract. Of interest is also the observation that the most marked proliferation in the vagina was usually noticeable in its proximal portion adjoining the cervix, while it was distinctly less marked in the distal portion.

In general those factors which intensify the proliferation of the vagina likewise tend to cause an earlier opening of this organ; this parallelism is noticeable as far as the graded effects of extracts of different potency are concerned. It is noticeable also in cases in which the effects of underfeeding are very marked and therefore the hypotypical changes in the ovaries very pronounced. The parallelism is apparent furthermore in animals with large persistent corpora lutea. In these cases the proliferation of the vaginal epithelium is inhibited and the opening of the vagina is delayed. However while this rule holds good in a general way, in individual cases there does not need to be always an exact parallelism between the amount of proliferation found in the vagina and the length of time required for its opening.

Of interest is also the fact that in the guinea pigs in which the injections were begun during the cycle and continued for a relatively long period of time, there took place an alternate closing and opening of the vagina occurring at relatively short intervals. On the other hand, this cyclic opening and closing seems to be lacking in castrated guinea pigs, notwithstanding the continued action of the extract; however, it seems desirable to extend the injections in castrated animals over a longer period of time before considering these differences in the opening and closing of the vagina in different series as definitely proven.

4. The effect of the injections of the follicular extract on the uterine mucosa is of special interest. In the first place we found that in the guinea pig the injections do not as a rule cause any marked proliferative changes in the uterus. They may call forth some hyperemia, and here and there an edematous condition of the mucosa, probably resulting from the hyperemia; but out of fifty-four injected animals, fifty-one distributed over all our series, did not show any growth processes in the mucosa, except the usual mitotic proliferation in the surface epithelium. In only three animals did we find marked heat changes consisting in a proliferation of the neck of the glands, enlargement of cells and nuclei and some mitotic division in the connective tissue of the mucosa. As stated above, these three guinea pigs had all been injected at about the same time with an extract which otherwise was not found to be especially potent. This activity in the mucosa is characteristic of a certain stage during the heat period, and it might therefore be expected to be observed whenever typical oestrus occurs under the influence of the injection. But in reality we have seen only a very few cases of this kind in these experiments. While as a rule the injections thus do not have any marked effect tending toward the initiation of growth processes in the uterine mucosa, in the underfed animals they may perhaps diminish the reduction in size of the uterine wall which otherwise would result from insufficient feeding.

Even if we add to the action of the extract a mechanical stimulation in the form of incisions into the uterine wall, the formation of placentomata or even a predeciduomatous proliferation does not take place. The follicular extract does not therefore call forth a sensitization of the uterine mucosa, such as is produced through the internal secretion of the corpus luteum. The contrast between the effect of the follicular extract on the vagina, on the one hand, and on the uterus, on the other hand, is thus very striking. But we can go even further and state that the injections of the follicular extract as a rule not only do not call forth predeciduomatous proliferation of the uterine mucosa, but that they prevent even the proliferation which would normally occur about five to seven days following ovulation, under the influence of the substances given off by the corpus luteum.

5. The injection of the follicular extract is in all cases associated with a

proliferation of the mammary gland, and this occurs in guinea pigs in which the gland consists merely of a system of ducts as, e.g., in immature animals, as well as in guinea pigs in which a formation of lobules and acini has taken place. As in our previous experiments we found also in these investigations especially large glands in hysterectomized guinea pigs in which, in addition to the action of the follicular extract, the large persisting corpus luteum had caused a marked proliferation in the mammary tissue. This effect of the follicular extract seems to correspond to the growth which occurs at the time of ovulation or directly preceding or following it and which is apparently due to substances given off by the mature follicles.

6. When we now inquire on the basis of the evidence which these investigations add to our previous data, into the relationship between the internal secretions of the corpus luteum and the follicular substance, we find a confirmation of our earlier conclusion, that the function of the corpus luteum is independent of the action of the follicular extract, and that the opinion recently expressed by Frank, Allen and others, that the follicular extract alone contains an ovarian hormone, while the corpus luteum acts merely as a depository for this follicular substance, which latter represents thus the only ovarian principle, has not been substantiated through these investigations. The effects of the internal secretions of the corpus luteum are in part quite distinct from those of the follicular extract, and when in other respects both produce similar effects it can be shown that the function of the corpus luteum is not merely due to a follicular substance which it has absorbed. Thus we have seen that the corpus luteum does not induce proliferation of the vaginal epithelium while the follicular extract has this effect in a very marked degree. On the other hand, the follicular extract does not cause the least sensitization of the uterine mucosa, nor, under the influence of mechanical stimulation, are placentomata produced in the uterine mucosa preceding and during continued injections of the extract. On the contrary, we found evidence pointing to the conclusion that the follicular extract prevents the proliferation in the uterine mucosa which otherwise would be normally produced by substances given off by the corpus luteum.

On the other hand, both corpus luteum and follicular extract may exert some similar effects. Both are able to cause proliferation of the mammary gland and this observation is in agreement with our previous analysis of the mechanism of the sexual cycle. In the guinea pig we observed a marked effect only of the persistent large corpus luteum, but not of the cyclic corpus luteum on the growth of the mammary gland; the cyclic corpus luteum has in the guinea pig, at best, only a very slight influence on this gland in contradistinction to its effect in the rabbit; in the latter animal Ancel and Bouin have previously observed a very rapid proliferation in the mammary gland under the influence of the cyclic corpus luteum. However, the effects on

the mammary gland on the part of the follicular substance and of the corpus luteum are quite distinct; the latter begins to act after the effect of the former has subsided.

On the other hand, we discovered a similarity between the action of the corpus luteum and the follicular extract, which was quite unexpected. We found that the inhibiting influence which the corpus luteum exerts normally on ovulation can also be obtained through the intermittent injection of follicular extract and that the injections of this extract must therefore necessarily lead to sterility of the animal, as long as these injections are continued. But in this case we have to deal with effects which are produced only under these experimental conditions and which are quite different from those obtaining during the normal ovarian cycle. In the normal guinea pig only the corpus luteum inhibits ovulation while there is no evidence that the follicular apparatus exerts such a function. There are other discrepancies which we noted between the action of the follicular substance under normal conditions and its effects under abnormal experimental conditions. In the first place, no autocatalytic acceleration of maturation of the follicles is observed in the normal cycle, while under certain experimental conditions the follicular extract may promote the growth of the follicles. And secondly, the intensity of growth in the vagina is much greater under normal conditions during the first phase of the sexual cycle than under experimental conditions. In the normal animal apparently a very small amount of the substance given off by the follicles may bring about a more active proliferation of the epithelium of the vagina than the relatively large amounts of the extract injected twice daily subcutaneously or intraperitoneally. And yet the latter inhibits ovulation, while the former does not have this effect. In this connection it is of interest to note that the recent observations of Parkes (Proc. Roy. Soc. B., 1927, ci, pp. 71 and 95) on the sexual cycle in the mouse subjected to Roentgen rays would suggest that the heat changes in this animal may not be due to the action of follicular substances but to another mechanism.

In this connection we may again point out a view expressed by one of us on previous occasions, namely, that the action of a small amount of hormone passing into the circulation constantly is not necessarily reproduced by the intermittent injection of relatively large amounts of the same substance. The results may therefore be different in the two cases. How far this or some other factor is responsible for the differences in effects noticed under normal conditions and under the conditions present in these experiments needs further investigation.

7. Lastly we must again emphasize the difference in the action of the cyclic small and of the persistent, large corpus luteum. The latter definitely inhibits the proliferation of the vaginal epithelium, and it seems to be responsible for the essential conditions characteristic of the pregnant

animal. This conclusion is borne out by the fact that the corpus luteum of hysterectomized guinea pigs has essentially the same effects as the corpus luteum of pregnancy. Whether the differences between the large persistent and the small cyclic corpora lutea are of a qualitative or of a quantitative character needs further investigation.

8. If we now inquire, on the basis of our data in the guinea pig, into the possible effects of injections of follicular extract in the human species, we may conclude that the chances seem to be very slight that here pronounced actions, similar to those observed in the small animals can be obtained with the limited quantities of this substance which it is possible to inject subcutaneously.

SUMMARY

1. Injections of follicular extract in immature guinea pigs accelerate the maturation of follicles; similarly in underfed guinea pigs they delay the hypotypical state of the ovaries or diminish the intensity of this process and thus allow the better development of the follicles as compared to the condition in undernourished, but not injected, controls. Otherwise injection of the follicular extract does not seem to accelerate noticeably the maturation of follicles or to increase the number of mature follicles; at least no distinct difference in this respect could be found between injected and not injected animals during the sexual cycle, after hysterectomy, in pregnancy and after castration. An acceleration of the maturation of follicles through a substance given off by growing and mature follicles,—a process which in certain respects would resemble an autokatalytic action —is not discernible in the normal animal.

2. Injections of follicular extract prevent ovulation in the guinea pig and must therefore lead to sterility as long as the injections are continued. This effect of the injections is not analogous to the action of the follicular substance in the normal animal; it must be due either to the abnormal mode of administration of the extract, to a substance admixed to it, or possibly to the lack of sufficient absorption of the follicular substance in the normal animal at the time of oestrus or preceding it.

3. The injections of follicular extract cause a proliferation of the epithelium of the vagina and its full development into squamous epithelium, corresponding to the changes which take place during the follicular phase of the normal cycle. These effects are the more pronounced, the stronger the extract is which is injected. However, the changes which occur normally in the vagina preceding or during heat are relatively greater than the effects produced by the injection of considerable quantities of follicular extract, although in the former case only very small amounts of the active substance can be absorbed. Whether this difference in the intensity of

action is due to differences in mode of administration of the active substance in the two cases or to other factors, is uncertain at the present time.

4. The large, persistent corpus luteum, as it exists in hysterectomized and pregnant animals, tends to counteract the accelerating effect of the injections on the opening of the vagina and the proliferation of the vagina under the influence of the injections. The antagonism to the opening of the vagina can be overcome through injection of a very active substance much more readily than the inhibition of growth. Proliferative processes in the epithelium of the vagina could not be induced in hysterectomized and pregnant animals through the injection of potent follicular extract; these two effects of the extract on the vagina can thus be dissociated experimentally. In the cyclic corpus luteum the inhibiting influence is either lacking or is much less pronounced, although both the large persistent, as well as the cyclic, corpus luteum, has the ability to prevent ovulation. These investigations thus confirm and bring out still more definitely the difference in the effects of the large, persistent corpus luteum on the one hand, and of the small cyclic corpus luteum on the other hand, which one of us observed previously. Whether this difference is of a qualitative or merely of a quantitative character, needs further investigations. In addition, these experiments confirm likewise our conclusion that this action of the large and persistent corpus is not limited to the state of pregnancy but is found equally in hysterectomized guinea pigs, and that the principal changes characteristic of the pregnant guinea pig are thus largely due to the specific effect of the large, persistent corpus luteum.

5. The injections of follicular extract have in general only very slight effects on the uterus of the guinea pig; they cause swelling, hyperemia, slight localized edema and some hemorrhage in the mucosa; in a few cases marked oestral changes in surface epithelium, neck of glands and connective tissue were observed. They may also retard the retrogression of the uterus caused by underfeeding or accelerate the growth of the uterus in immature guinea pigs. However, they do not produce the sensitization of the uterine mucosa to mechanical stimuli leading to the development of placentomata, while on the other hand such a sensitization takes place readily under the influence of a hormone given off by the corpus luteum. Neither does the follicular substance cause the predecidual proliferation of the uterine mucosa, which is produced by the corpus luteum at a certain phase of the normal cycle, but, on the contrary, it tends to prevent this effect. On the other hand, the corpus luteum does not cause the proliferation of the vagina which is called forth by the follicular substance; it tends rather to prevent this effect.

6. The follicular extract induces proliferative processes in the mammary gland, which correspond to similar growth processes taking place normally in the follicular phase of the sexual cycle in the guinea pig.

7. We may therefore conclude that the ovarian follicles and corpus luteum contain or give off substances which are not identical. Some of the effects of these substances on the sex organs are different, while others are similar. We may thus consider the corpus luteum as a gland with an internal secretion of its own and we may furthermore conclude that the effectiveness of this organ does not depend upon the receiving and storing up of substances given off by the follicles.

BIBLIOGRAPHY

(1) LOEB, L. Biol. Bull., 1914, xxvii, 1.
(2) LOEB, L. Trans. Amer. Gyne. Soc., 1917; Surg., Gynec., and Obstet., 1917, xxv, 300.
(3) LOEB, L. Amer. Journ. Anat., 1923, xxxii, 305.
(4) FRANK, R. T. Journ. Amer. Med. Assoc., 1922, lxxviii, 181; Journ. Obst. and Gynec., 1924, viii, 573.
FRANK, R. T. AND R. G. GUSTAVSON. Journ. Amer. Med. Assoc., 1925, lxxxiv, 1715.
(5) ALLEN, E. Amer. Journ. Anat., 1922, xxx, 297.
ALLEN, E., J. P. PRATT AND E. A. DOISY. Journ. Amer. Med. Assoc., 1925, lxxxiv, 399.
ALLEN, E., B. F. FRANCIS, C. C. ROBERTSON, C. JOHNSON, E. A. DOISY, W. B. KOUNTZ AND H. GIBSON. Amer. Journ. Anat., 1924, xxxiv, 133.
(6) STOCKARD, C. R. AND G. N. PAPANICOLAOU. Amer. Journ. Anat., 1917, xxii, 255.
(7) LOEB, L. Deutsch. Med. Wochenschr, 1911, xxxvii, 17; Journ. Morphol., 1911, xxii, 37.
(8) MARSHALL, F. H. A. AND E. T. HALMAN. Proc. Roy. Soc. Ser. B., 1917, lxxxiv, 546.
(9) LOEB, L. Journ. Amer. Med. Assoc., 1907, l, 1809; Zentralbl. f. Allg. Path., 1908, xviii, 563; Arch. f. Entwmech., 1909, xxvii, 89, 463; Ibid., 1911, xxxi, 456; 1911, xxxii, 67.
(10) DRUMMOND, ROBINSON AND ASDELL. Journ. Physiol., 1926, lxi, 608.
(11) PARKES, A. S. AND C. W. BELLERBY. Journ. Physiol., 1926, lxii, 145.
(12) ANCEL, P. ET P. BOUIN. Journ. Physiol. et Path. Gen., 1911, xiii, 31.
LOEB, L. AND C. HESSELBERG. Journ. Exper. Med., 1917, xxv, 285, 305.
(13) LOEB, L. Biol. Bull., 1915, xxviii, 59.
(14) LOEB, L. Biol. Bull., 1917, xxxii, 91.
(15) LOEB, L. Proc. Soc. Exper. Biol. and Med., 1923, xx, 441.
(16) PAPANICOLAOU, G. N. AND C. R. STOCKARD. Proc. Soc. Exper. Biol. and Med., 1920, xvii, 143.
(17) LOEB, L. Science, 1917, xlv, 59.
WALSH, L. S. N. Journ. Exper. Med., 1917, xxvi, 245.

THE HEMODYNAMIC ACTION OF MINIMAL EFFECTIVE DOSES OF EPINEPHRIN IN THE UNANESTHETIZED DOG

CARL A. DRAGSTEDT, ARDATH H. WIGHTMAN AND JOHN W. HUFFMAN

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois

Received for publication November 25, 1927

One of the chief arguments against the tonus theory of medulliadrenal function and one that is held also to controvert any emergency function is chiefly a quantitative one, e.g., that epinephrin is not secreted in amounts comparable to those that are necessary to produce its pharmacological effects. The epinephrin effect that has long held the chief interest is that of its effect on blood pressure. Accurate determinations, therefore, of the minimal effective dose of epinephrin are necessary. Hoskins and McClure, using dogs anesthetized with ether, determined the minimal effective dose as approximately 0.42 cc. of a 1 to 1 million solution of adrenalin per kilo per minute. This they found to give a fall of pressure and that it required some five times the amount to cause a rise. Inasmuch as they corroborated the findings of Moore and Purinton that the minimal effective dose caused a fall in pressure there seemed to be little hope that the figures for the minimal pressor amounts could be further lessened and apparently for this reason there have been no subsequent studies of this nature.

There have been numerous studies since, however, which justify a reopening of this question. Collip (1921) reported that the depressor effect of a small dose of adrenalin in an animal under light ether or chloroform anesthesia may be converted into a pure pressor effect by increasing the anesthetic, and that decreasing the anesthetic may restore the depressor effect. Wyman and Lutz (1925a) noted that when ether was used as an anesthetic in cats, prior to pithing, the pressor responses to equal doses of adrenalin progressively increased in height for about an hour after discontinuing the ether. Then the responses decreased in height for a time and finally became of equal magnitude. A large dose of ether would then cause a marked decrease in the height of the pressor response to a given dose of adrenalin (Lutz and Wyman, 1925b). Cannon and Lyman (1913) had earlier reported that the depression of blood pressure seen with small doses of adrenalin in anesthetized cats is changed to a rise in pressure with the same dose after the animal is pithed. They

stated that "pithing has transformed the vasodilator action of adrenalin to a vasoconstrictor action." This transformation could just as well be associated with the withdrawal of the anesthetic as with injury to the central nervous system. Lieb and Hyman (1922a, b, c, d) reported finding variable responses to equal doses of adrenalin in pithed cats which they attributed to a sensitization of the involuntary nervous system, probably at the myoneural junction. Burget and Visscher (1927) deny that there is such a sensitization and showed that the adrenalin response of the vascular system of the pithed cat may be made to vary at any time by varying the pH of the blood. They found that from pH 6.9 to 8.0 the response progressively increases. Luckhardt and Koppanyi (1927) in their studies on the hemodynamic effect of subcutaneously injected epinephrin found that a pressor response was not readily obtained in animals under barbital after massage of the injected area whereas good responses were obtained under paraldehyde. They found ether to diminish or entirely suppress the pressor response both when administered by inhalation and when injected intravenously in small doses. Morphine apparently had no effect.

In view of these findings it seemed necessary to attempt the study of the hemodynamic action of epinephrin in the unanesthetized dog. A preliminary report has recently appeared (Dragstedt and Wightman, 1927).

GENERAL EXPERIMENTAL METHODS. 1. *Preparation of animals.* Healthy, adult dogs both male and female were used. In some experiments the animals were prepared by transplanting one carotid artery externally to the skin under ether anesthesia and with aseptic precautions. This was done by making a curved incision in the neck so that the skin flap could be carried under the artery and the suture line would not lie beneath or in contact with the vessel. The artery was then coated with vaseline and the neck carefully bandaged to avoid injury during the animal's recovery from the anesthetic. Such animals are suitable for use on the following day or for as long a period as one week. Owing to the possibility of thrombosis, however, the animals were usually used either 24 or 48 hours after the operation. These animals were comfortably placed on a table and soothed by attendants so that the carotid cannulae could be inserted without difficulty and with no pain to the animal. A hypodermic needle was then inserted into the saphenous vein for the injections. In a few experiments the saphenous vein was exposed under local anesthesia and cannulated. This introduced the factor of possible cocaine sensitization so that this method was only used in a few experiments. In the majority the spinal cord was transected in the lower dorsal region at the time of the carotid transplantation so that exposure and cannulation of the leg veins could be made without local anesthesia and with no disturbance of the animal. In such animals

there was also the advantage that they were less likely to damage the artery transplant by clawing at the bandage with their hind feet, and they were also more prone to lie quietly during the subsequent experiment. In another series of experiments, animals were anesthetized with ether long enough to insert carotid and vein cannulae and were then allowed to recover from the ether under morphine or morphine and

TABLE 1

Table showing the hemodynamic effect of epinephrin in unanesthetized and undrugged dogs

DOG NUMBER	WEIGHT	EPINEPHRIN OF 1–1,000,000 PER KILO PER MINUTE	DURATION OF INJECTION	BLOOD PRESSURE RESPONSE
	kilos	*cc.*	*minutes*	
4	11.0	0.37	2.0	Sustained rise of 18 mm. Hg
7	7.0	0.33	2.0	Sustained rise of 8 mm. Hg
10	8.0	0.39	2.0	Sustained rise of 16 mm. Hg
13	19.4	0.31	2.0	Sustained rise of 12 mm. Hg
14	11.0	0.36	2.5	Sustained rise of 10 mm. Hg
15	15.0	0.34	2.0	Sustained rise of 6 mm. Hg
16	14.5	0.28	2.0	Sustained rise of 14 mm. Hg

TABLE 2

Table showing the hemodynamic effect of epinephrin in dogs after recovery from ether under morphine, atropine or both

DOG NUMBER	WEIGHT	HOURS AFTER ETHER	MORPHINE SULPHATE	ATROPINE SULPHATE	EPINEPHRIN OF 1–1,000,000 PER KILO PER MINUTE	DURATION OF THE INJECTION	BLOOD PRESSURE RESPONSE
	kilos		*mgm.*	*mgm.*	*cc.*	*minutes*	
2	11.1	1.5	15	1.0	0.36	2	Sustained rise of 18 mm. Hg
3	9.0	6	15	1.0	0.18	2	Sustained rise of 12 mm. Hg
6	14.6	3	30		0.75	2	Sustained rise of 15 mm. Hg
8*	12.0	3			0.56	2	Sustained rise of 4 mm. Hg
9	10.0	4	30	1.0	1.2	2	Sustained rise of 12 mm. Hg

* This dog has shown sustained falls of blood pressure while under ether with doses from 0.3 to 2.6 cc. of 1–1,000,000 epinephrin per kilo per minute.

atropine administered in sufficient amounts to maintain a very light narcosis.

2. *Preparation of solutions.* Pure crystalline adrenalin furnished by Parke, Davis & Company was used. Stock solutions of 1 to 5000 strength were prepared in freshly boiled, slightly acidified, distilled water every week, and dilutions were made from such stock solutions in distilled water just prior to use. A marked deterioration, even within the period

of one hour, was noted when the higher dilutions were made in Ringer's solution.

3. *Method of injections.* Injections were made into the saphenous or femoral vein by means of a Woodyatt pump. The stroke volume of the pump was repeatedly checked and the stroke rate constantly timed so that no errors in the total volume injected because of variations in the line voltage could occur and the variations in the rate of the injection were negligible. Injections were made over periods of 2, 3 or 4 minutes and the total volume of fluid injected in this time was from 4 to 10 cc. The amount of adrenalin injected was varied by changing the concentration of the solution and to a lesser degree by altering the rate of the pump. Control injections of distilled water, normal saline and Ringer's solution were frequently made.

RESULTS. In all the experiments so far performed on the unanesthetized dog (to date 16), the minimal effective dose of epinephrin was found to produce a pressor effect on the blood pressure. The actual minimal effective dose is difficult to state because minor variations in the blood pressure tracing of the unanesthetized dog cannot always be discerned with accuracy. It has been expressed therefore as being less than some dose that has produced an unequivocal sustained rise impossible to confuse with any normal variation. In tables 1, 2 and 3 the pressor responses seen after more than minimal doses are expressed in millimeters of mercury and in table 4 there is a summary indicating the minimal effective dose. Figures 1 to 6 illustrate some of the results obtained. From the results obtained it can be definitely stated that in the undrugged, unanesthetized dog, epinephrin injected at rates of from 0.2 cc. to 0.4 cc. of a 1 to 1,000,000 solution (0.0002 to 0.0004 mgm.) per kilo per minute will produce a sustained rise of blood pressure throughout the

Fig. 1. Expt. 16 (4). Male dog, 14.5 kilos. Carotid transplanted and spinal cord transected below thoracic XII. No drugs or anesthetic for twenty-four hours. *A* to *B*, 0.5 cc. 1 to 1,000,000 adrenalin per kilo per minute for 2.8 minutes.

Fig. 2. Expt. 3 (53). Male dog, 9 kilos. Six hours after ether. Morphine sulphate 15 mgm., atropine sulphate 1 mgm. *A* to *B*, 0.22 cc. 1 to 1,000,000 adrenalin per kilo per minute for 2 minutes.

Fig. 3. Expt. 2 (24). Male dog, 11 kilos. Three hours after ether, morphine sulphate 15 mgm., atropine sulphate 1 mgm. *A* to *B*, 0.5 cc. Distilled water per kilo per minute for 2 minutes.

Fig. 4. Expt. 2 (25). Same as figure 3. *A* to *B*, 0.43 cc. 1 to 1,000,000 adrenalin per kilo per minute for 2 minutes.

Fig. 5. Expt. 10 (2). Male dog, 8 kilos. Carotid transplanted and spinal cord transected below thoracic XII. No drugs or anesthetic for 24 hours. *A* to *B*, 0.53 cc. 1 to 1,000,000 adrenalin per kilo per minute for 2 minutes.

Fig. 6. Expt. 10 (12). Same as figure 5. Fifteen milligrams cocaine hydrochloride given prior to injection. *A* to *B*, 0.20 cc. 1 to 1,000,000 adrenalin per kilo per minute for 2 minutes.

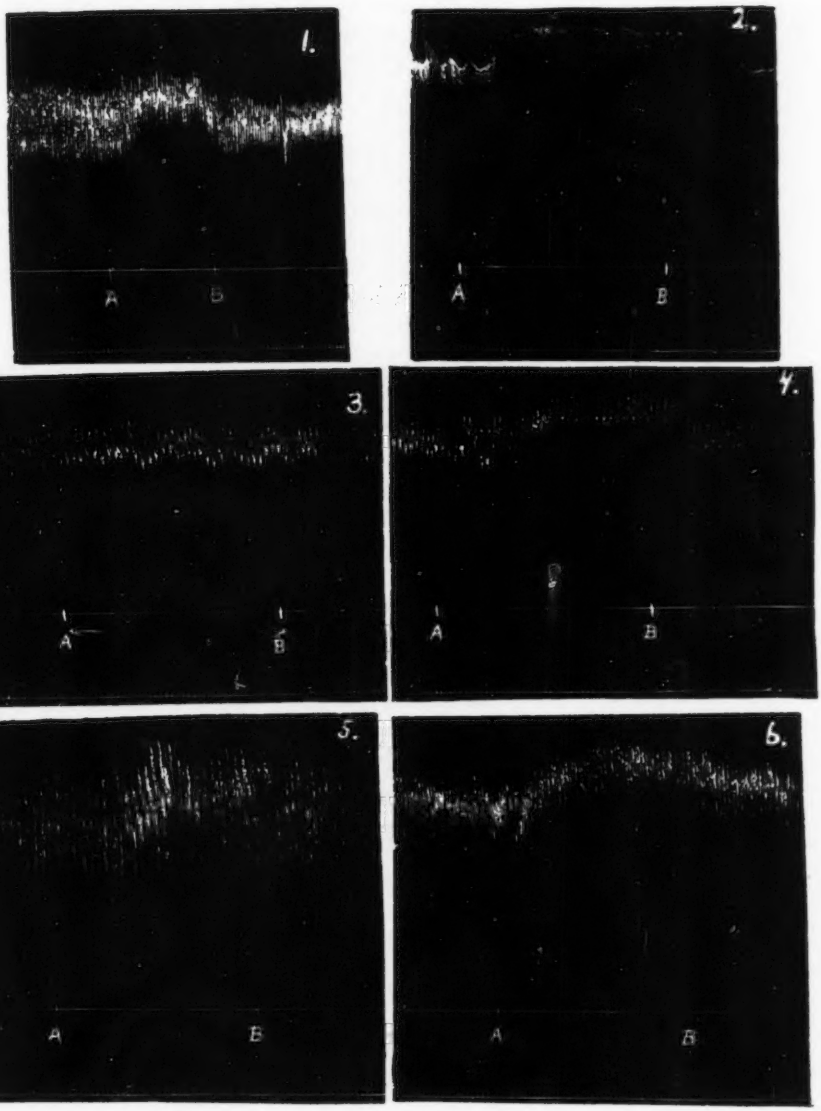

1.
A
B
2.
A
B
3.
A
B
4.
A
B
5.
A
B
6.
A
B

TABLE 3

Table showing the hemodynamic effect of epinephrin in unanesthetized dogs to which morphine, atropine or both have been administered

DOG NUMBER	WEIGHT	MORPHINE SULPHATE	ATROPINE SULPHATE	EPINEPHRIN OF 1–1,000,000 PER KILO PER MINUTE	DURATION OF THE INJECTION	BLOOD PRESSURE RESPONSE
	kilos	*mgm.*	*mgm.*	*cc.*	*minutes*	
1	11.5		1.5	0.20	2	Sustained rise of 28 mm. Hg
11	13.0	30		0.44	2	Sustained rise of 12 mm. Hg
12	18.5	15		0.40	2	Sustained rise of 12 mm. Hg
13	19.4	15	1.0	0.51	3	Sustained rise of 10 mm. Hg
14	11.0	15		0.30	3	Sustained rise of 10–14 mm. Hg

TABLE 4

DOG NUMBER	WEIGHT	SEX	MORPHINE SULPHATE	ATROPINE SULPHATE	HOURS AFTER ETHER	MINIMAL EFFECTIVE (PRESSOR) DOSE OF EPINEPHRIN OF 1–1000,000 SOLUTION PER KILO PER MINUTE	REMARKS
	kilos		*mgm.*	*mgm.*		*cc.*	
1	11.5	F.		1.5	24	Less than 0.20	Carotid transplant. Vein exposed under local
2	11.1	M.	15.0	1.0	1.5	Less than 0.35	
3	9.0	M.	15.0	1.0	6	Less than 0.12	
4	11.0	M.			24	Less than 0.28	Carotid transplant
6	14.6	M.	30.0		3	Less than 0.30	
7	7.0	F.			24	Less than 0.25	Carotid transplant. Spinal cord cut
8	12.0	F.			3	Less than 0.56	
9	10.0	M.	30.0	1.0	4	Less than 0.80	
10	8.0	M.			24	Less than 0.39	Carotid transplant. Spinal cord cut
11	13.0	M.			24	Less than 0.44	Carotid transplant. Spinal cord cut
12	18.5	M.	15.0	1.0	24	Less than 0.32	Carotid transplant. Spinal cord cut
13	19.4	M.			24	Less than 0.31	Carotid transplant. Spinal cord cut
14	11.0	M.			24	Less than 0.30	Carotid transplant. Spinal cord cut
15	15.0	M.			24	Less than 0.34	Carotid transplant. Spinal cord cut
16	14.5	M.			24	Less than 0.28	Carotid transplant. Spinal cord cut

period of injection. Neither morphine nor atropine appear materially to influence the result except insofar as they make the experiment easier to do and the blood pressure tracing easier to read. Definite synergism with cocaine can be shown. For example, the response seen in figure 6 to 0.2 cc. 1 to 1 million adrenalin per kilo per minute is more prompt in appearance and more definite than the response in figure 5 to 0.53 cc. Some data have been collected with regard to the degree and duration of such synergistic effects when cocaine is administered intravenously or subcutaneously as in its use in local anesthesia, in order to control the results in the few experiments in which veins were exposed under local anesthesia, but comment on this is reserved for a later date.

DISCUSSION. Stewart and Rogoff (1920) give the average output of epinephrin by the adrenals in the dog as 0.00022 mgm. per kilo of body weight per minute which is the equivalent of 0.22 cc. of a 1 to 1 million solution of the base. The results here reported show that this amount or one but from 50 per cent to 100 per cent greater produces pressor effects in the unanesthetized dog and are not in harmony with the statement by Hoskins (1927) that it requires twenty times the normal output to produce pressor effects. A discussion as to whether this variation in output can occur under physiological conditions and the significance, therefore, of these findings does not fall within the scope of this article.

CONCLUSIONS

1. The minimal effective dose of epinephrin on sustained administration in the unanesthetized dog produces pressor effects.

2. The minimal effective dose of epinephrin in the unanesthetized dog is less than from 0.2 to 0.4 cc. of a 1 to 1 million solution of adrenalin per kilo per minute.

BIBLIOGRAPHY

BURGET, G. E. AND M. B. VISSCHER. 1927. This Journal, lxxxi, 113.
CANNON, W. B. AND H. LYMAN. 1913. This Journal, xxxi, 376.
COLLIP, J. B. 1921. This Journal, lv, 450.
DRAGSTEDT, C. A. AND A. H. WIGHTMAN. 1927. Proc. Soc. Exper. Biol. and Med., xxv, 22.
HOSKINS, R. G. 1927. Journ. Amer. Med. Assoc., lxxxviii, 2011.
HOSKINS, R. G. AND C. W. MCCLURE. 1912. Arch. Int. Med., x, 343.
LIEB, C. C. AND H. T. HYMAN. 1922a. This Journal, lxiii, 60.
1922b. This Journal, lxiii, 68.
1922c. This Journal, lxiii, 83.
1922d. This Journal, lxiii, 88.
LUCKHARDT, A. B. AND T. KOPPANYI. 1927. This Journal, lxxxi, 436.
WYMAN, L. C. AND B. R. LUTZ. 1925a. This Journal, lxxii, 488.
1925b. This Journal, lxxiii, 254.
STEWART, G. N. AND J. M. ROGOFF. 1920. This Journal, lii, 521.

FEEDING EXPERIMENTS WITH PLANTS AT DIFFERENT STAGES OF DEVELOPMENT

III. Synthesis of Vitamin in Corn[1]

MAXWELL KARSHAN, FRANCES KRASNOW AND BENJAMIN HARROW

From the Biochemical Laboratory of Columbia University at the College of Physicians and Surgeons, New York City

Received for publication November 26, 1927

We have already pointed out (1) that in order to determine whether there is a synthesis of vitamin during germination and greening of plant material, it is not enough to compare *equal weights* of the *dry material* (ungerminated, germinated or green corn), but that an *equal number* of seeds should be compared. We have adopted this procedure.

The methods employed have been given in detail in earlier publications (2), but as certain modifications were employed, these will be discussed briefly. The seeds (corn) were dried, counted, weighed and ground. Knowing the number of seeds and their weight, the average weight of each seed could be calculated. One such experiment is summarized in the following table:

	UNGERMINATED	GERMINATED	GREEN SEEDLINGS
Number of seed	3782	3782	2848
Total weight	631 grams	599 grams	315 grams
Weight per seed	0.1666 gram	0.1588 gram	0.1106 gram*

* The decrease in the weight of the seedling, as compared with the weights of the germinated and ungerminated corn, is explained by the obvious fact that a seedling uses up rapidly some of the stored material accumulated in the seed and for a time loses a great deal in dry weight, which it regains with further growth.

Each rat was fed an amount equivalent to six seeds per day. It will at once be noticed that though the *number of seeds offered is the same, the weight is not the same* in all cases.

Experiments were undertaken to determine the possible synthesis of vitamins A and B. In tests for the synthesis of vitamin A, the rats were first supplied with a synthetic mixture (casein 18 grams, corn starch 54

[1] A preliminary report of this work appeared in the Proceedings of the Society for Experimental Biology and Medicine, 1927, xxiv, 765.

grams, fat (crisco) 24 grams, salt mixture[2] 4 grams and water, 300 cc.) to which was added dried yeast to supply vitamin B. Each rat received 0.2 gram of yeast per day. This was mixed with 5 grams of synthetic mixture and was fed separately, no more synthetic mixture being offered until all visible particles had been consumed. This diet was continued until the animals began to lose weight; then corn was added.

When the factor to be tested was vitamin B, the same dietetic schedule was maintained except that, instead of feeding yeast, vitamin A was supplied in the form of cod-liver oil, each rat receiving 1 gram per day. When the animals began to lose weight, corn was added to the diet.

TABLE 1

Tests for vitamin A

	RAT NUMBER	TOTAL NUMBER OF DAYS FED ON CORN	TOTAL GAIN OR LOSS IN WEIGHT	GAIN OR LOSS PER DAY
			grams	*gram*
Ungerminated corn	1	79	−2	−0.01
	2	40	−18	−0.45
	3	58	−6	−0.10
	4	66	−1	−0.02
	5	53	−27	−0.51
Germinated corn	6	76	−25	−0.33
	7	63	−19	−0.30
	8	62	−24	−0.34
	9	79	−9	−0.11
	10	56	−27	−0.48
Green seedlings	11	79	+13	+0.16
	12	79	+15	+0.19
	13	79	+15	+0.19
	14	79	+8	+0.10
	15	79	+21	+0.23

As already mentioned, the corn was added in amounts equivalent to six seeds, mixed with 5 grams of synthetic mixture plus either the 0.2 gram yeast or 1 gram cod-liver oil. Not until all this was consumed was any more synthetic mixture given.

Each rat was kept in a separate cage. The groups of rats were biologically balanced, that is, rats 1-6-11 were of the same litter. This family relationship holds for rats 2-7-12, 3-8-13, 4-9-14, 5-10-15 (table 1) and

[2] The salt mixture consisted of tricalcium phosphate 10 grams, dipotassium hydrogen phosphate 37 grams, sodium chloride 20 grams, sodium citrate 15 grams, magnesium citrate 8 grams, calcium lactate 8 grams and ferric citrate 2 grams.

A-E-I, B-F-J, C-G-K, D-H-L (table 2). The results may be summarized as follows:

All the rats on germinated and ungerminated corn not only lost weight but developed xerophthalmia sooner or later, and eight of the ten rats died between the 40th and 79th day. Those on the green seedlings gained in weight, none of them developed the eye disease and there were no deaths. Since vitamin A is associated with xerophthalmia, we conclude that there is a definite synthesis of vitamin A during the course of the greening of corn. X-ray pictures of bones from rats 1, 9, 11, 12 did not show any signs of rickets.

The tests for vitamin B obviously point to an abundant supply of this vitamin in all three varieties of corn, but there is no indication of any increase in the amount of vitamin during the course of germination or greening.

TABLE 2

Tests for vitamin B

	RAT NUMBER	TOTAL NUMBER OF DAYS FED ON CORN	TOTAL GAIN IN WEIGHT	GAIN PER DAY
			grams	*grams*
Ungerminated corn	A	Did not reach stationary weight		
	B	47	90	1.9
	C	70	76	1.1
	D	70	103	1.4
Germinated corn	E	56	96	1.7
	F	78	98	1.5
	G	70	84	1.2
	H	70	106	1.5
Green seedlings	I	56	84	1.5
	J	62	102	1.6
	K	56	64	1.1
	L	47	60	1.3

SUMMARY

1. In experiments with corn it is shown that there is a definite synthesis of vitamin A during the course of greening.

2. All varieties of corn (ungerminated, germinated and green seedlings) contain vitamin B, but there is no evidence of any increase in the amount of this vitamin during the course of germination or in the formation of the green seedlings.

BIBLIOGRAPHY

(1) HARROW, B. AND F. KRASNOW. 1923. Journ. Met. Res., iv, 491.
1924. Proc. Soc. Exper. Biol. and Med., xxi, 232.
(2) HARROW, B. AND F. KRASNOW. 1922. Journ. Met. Res., ii, 401.

MOVEMENTS OF THE CECUM

WALTER C. ALVAREZ AND ARNOLD ZIMMERMANN

From the Division of Medicine, Mayo Clinic and The Mayo Foundation, Rochester, Minnesota

Received for publication November 28, 1927

During the last year, while taking motion pictures of the stomach and bowel of rabbits opened under a bath of salt solution, we have obtained many films showing the movements of the cecum. Some of these cecums were so unusually active, probably on account of the presence of diarrhea-producing parasites, that we thought it worth while to make a few analyses of the movements. This was done by measuring in successive pictures the distances between the transverse markings of the cecum and then plotting these measurements as ordinates with intervals of time as abscissas. As a result we obtained a series of curves such as are shown in the illustrations. These curves throw much light on the nature of the movements which ordinarily are too complex to be analyzed by the unaided eye. They show that not infrequently the waves in different parts of the cecum are running simultaneously in different directions, some toward the tip and some toward the caput where it joins with the ileum and colon. This accounts for the peculiar to-and-fro or wave-like motion that is often seen.

The rate of conduction appears to vary from moment to moment, and not infrequently the contractions fail to travel at all; the result is that it is often impossible to trace any influence of a contraction in one segment on those of the adjacent ones. Similarly, in the stomach of the dogfish, some strong contractions progress slowly for short distances, others travel rapidly for long distances, and others stay in one spot until they fade out. It suggests the presence either of a synaptic nervous mechanism or of refractory states in the muscle. We are accustomed to the idea of blockage in nervous paths but it is not so easy to see why the impulse should not spread from muscle fiber to muscle fiber. In figure 1 it can be seen that at one point the rate of conduction suddenly increased so that two segments contracted almost simultaneously, and then, as suddenly, the rate became slower than it had been at first. In the third record, at *S*, a long section of the cecum contracted systolically, indicating the presence of rapid conduction of some stimulus.

The second record in figure 1 shows that ordinarily, when the cecum is lying more or less dormant for minutes at a time, there is little sign of any

correlation between the movements of adjacent segments; the amplitude of contraction is small and the rhythm uneven. In such cecums, traveling waves seldom pass through more than three segments, and the organ becomes active only at long intervals, as when a peristaltic rush is forcing material through the ileocecal sphincter and on down the colon. Figure 2

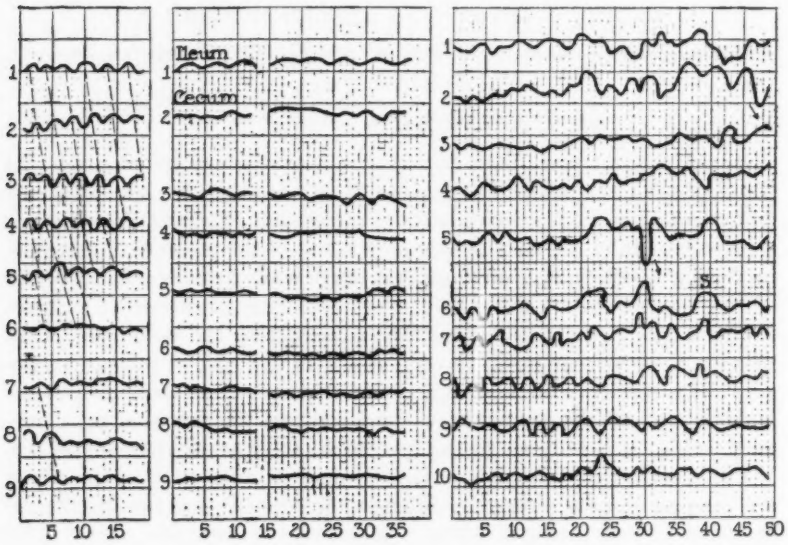
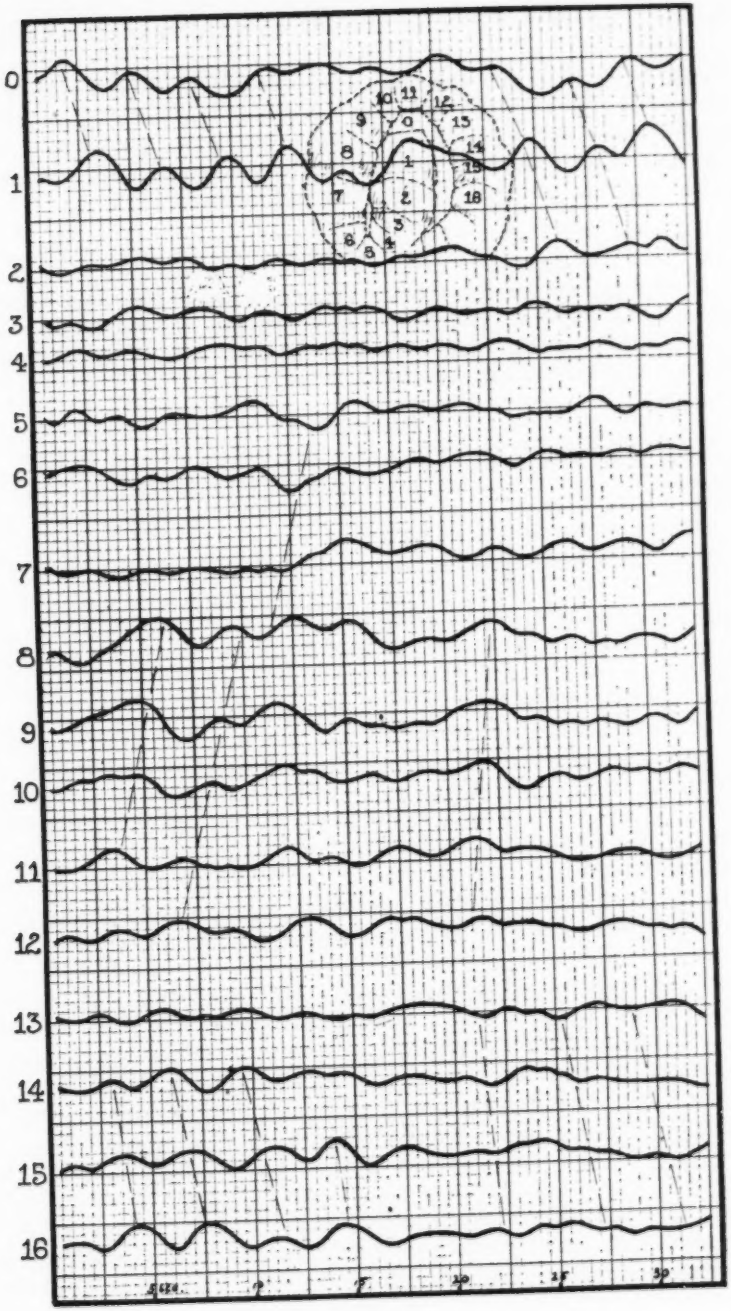


Fig. 1. Three records made by plotting as ordinates with intervals of time as abscissas, the distances between transverse markings on the cecums of rabbits, as shown in every sixteenth picture in a cinematographic film. The positions of the various segments of the cecum are shown in the small diagram in figure 2. In the second part of the figure the first record is from the terminal ileum; the rest are from the cecum. The figures at the bottom denote seconds. The dotted lines indicate direction of peristaltic progress. A rise in the curve indicates contraction of the longitudinal muscle.

illustrates the behavior of a cecum which was very active and in which conduction in both directions seemed to be good.

As Alvarez and Starkweather (1918) pointed out, the neuromuscular apparatus of the cecum should probably be much more sluggish than that of the small bowel so that it will not respond to many stimuli but will lie quietly, holding its contents with a firm and steady grip. Actually the

Fig. 2. Simultaneous records of the activity of the longitudinal muscle in seventeen segments of the cecum of a rabbit with diarrhea. The time record represents intervals of five seconds. The small drawing shows the location of the different segments, the activities of which were recorded photographically.


0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

muscle from the cecum has little tendency to beat rhythmically when excised from the body; it is insensitive to stimuli that are effective for the small intestine, it has a long latent period, and a long low curve of contraction. A brief resumé of the literature on the movements of the cecum will be found in the article by Alvarez and Starkweather.

SUMMARY

An analysis of motion pictures of the movements of the rabbit's cecum shows that much of the time there is little coördination between the movements of different segments. Occasionally there is a wave that travels first in one direction and then in the other.

BIBLIOGRAPHY

ALVAREZ, W. C. 1926. This Journal, lxxx, 493.
ALVAREZ, W. C. AND E. STARKWEATHER. 1918. This Journal, xlvi, 563.

STUDIES ON VIGOR

XV. The Effect of Thyroidectomy on Spontaneous Activity in the Rat, With a Consideration of the Relation of the Basal Metabolism to Spontaneous Activity

M. O. LEE and E. F. Van BUSKIRK

From the Laboratory of Physiology, The Ohio State University

Received for publication December 5, 1927

In view of the well-known regulatory effect of the thyroid on metabolic and growth processes, it was considered desirable to study the rôle of this gland in bodily vigor, as measured by the spontaneous activity of the white rat. Hoskins (1927), in a previous paper, has reported the effect on activity of thyroid administration in senility. The present paper reports, in part, the results secured by the experimental ablation of the thyroid gland, and, in part, various experimental evidence bearing on the relationship between basal metabolic rate and spontaneous activity.

Methods. Revolving cages, of the type described by Durrant (1924), were used to measure the spontaneous activity of the animals. The revolutions of the cylinders were additively recorded by automatic counters, and were tabulated daily. The adequacy of the method as a measure of activity and as a criterion of bodily vigor has been discussed by Durrant (1924) and Hoskins (1925).

Twenty-seven rats, thirteen males and fourteen females, which survived until the completion of the work, furnished the data for this study. Their ages, at the beginning of the experiment, varied from 93 to 160 days. All of the animals were kept in the activity cages for a preliminary period of from 35 to 50 days, in order to determine their normal individual activity levels. Rats of this age, after becoming accustomed to the cages, ordinarily manifest a rather high and rather constant level of activity for a period of several months, as we have repeatedly found in this laboratory. At the end of the preliminary period the rats were divided into two groups, of eighteen experimental and nine control animals, respectively, matched as nearly as possible in activity performance. Those animals designated as controls served only to demonstrate that no change in environmental conditions or other general factors occurred, which might vitiate the results. For the purpose of the study the experimental animals served as their own controls, inasmuch as the activity level after thyroidectomy was compared with the level for the 30 days immediately preceding the removal of

the glands. During the 30-day preliminary period the average daily activity of the experimental males was 2220 revolutions, of the control males 2398 revolutions, of the experimental females 6502 revolutions and of the control females 6788 revolutions (see tables 1 and 2). Thus, in both series, there was a slight difference in activity in favor of the controls. After the ablation of the thyroids the rats were kept in the revolving cages for a period of three months or, in three cases, until death occurred.

TABLE 1

Showing voluntary activity of control rats

RAT NUMBER	AGE AT END OF PRELIMINARY 30-DAY PERIOD	AVERAGE DAILY ACTIVITY				TOTAL AVERAGE DAILY ACTIVITY OF LAST 90 DAYS
		Preliminary 30-day period	First 30 days	Second 30 days	Third 30 days	
(a) Control males						
	days	*revolutions*	*revolutions*	*revolutions*	*revolutions*	*revolutions*
4092	153	1308	655	327	325	436
4117	142	744	242	465	934	547
4139	126	1332	2250	1460	1501	1734
4121	143	5785	4384	3644	4813	4280
4125	146	2823	4095	2500	2891	3162
Average....	142	2398	2325	1675	2093	2032
Per cent change from preliminary period.....			−3	−30	−12	−15
(b) Control females						
4133	153	7145	6322	5539	6443	6101
4149	128	7952	5830	4870	4483	5061
4122	158	7307	9054	9694	5952	7900
4131	153	4746	4907	5060	4699	4889
Average....	148	6788	6528	6291	5394	5988
Per cent change from preliminary period.....			−4	−7	−20	−12

Careful attention was paid to environmental conditions and to dietary requirements. Males and females in the activity cages were kept in separate rooms, but otherwise the conditions were similar and were relatively constant throughout the experiment. Except in the case of three males which died in the third month following thyroidectomy, the health of all of the animals appeared generally normal, although such signs of hypothyroidism as thinning and falling hair were present. The rats had access to food and water at all times. The ration was a McCollum

mixture supplemented with fresh cabbage and corn once a week, and is thought to be adequate in all dietary respects.

TABLE 2

Showing voluntary activity of thyroidectomized rats

RAT NUMBER	AGE AT THYROIDECTOMY	AVERAGE DAILY ACTIVITY 30 DAYS BEFORE THYROIDECTOMY	AVERAGE DAILY ACTIVITY FOLLOWING THYROIDECTOMY			TOTAL AVERAGE DAILY ACTIVITY AFTER THYROIDECTOMY
			First 30 days	Second 30 days	Third 30 days	
(a) Thyroidectomized males						
	days	*revolutions*	*revolutions*	*revolutions*	*revolutions*	*revolutions*
4093	153	620	312	241	358	304
4099	152	1399	1503	1258	escaped	1379
4116	141	1364	1466	2083	died	1775
4124	159	6558	4907	6369	5739	5672
4120	143	2575	2779	3622	died	3201
4128	146	392	718	417	1431	855
4132	154	1484	1585	937	1206	1243
4159	123	3360	2857	1014	died	1936
Average....	145	2220	2016	1992	2082	2046
Per cent difference from controls.....		−8	−14	+18	−1	+1
Per cent change from preliminary period			− 9	−10	−6	−8
(b) Thyroidectomized females						
4126	159	3397	2454	4901	3636	3664
4119	149	6516	4169	4306	4148	4658
4129	152	7971	6374	7206	8236	7283
4137	137	8124	6710	8543	8115	7789
2326	174	3087	3175	3361	3284	3273
2319	143	12520	9432	12877	10684	10998
2323	190	2532	1912	2772	2350	2345
2300	182	1421	1227	2046	1633	1635
2316	161	10223	10064	9771	9110	9648
2919	146	4530	3754	5108	4067	4310
Average....	159	6032	4927	6089	5526	5560
Per cent difference from controls.....		−11	−24	−3	+2	−7
Per cent change from preliminary period			−18	+1	−8	−8

The thyroid glands were removed according to the technic previously described by one of us (Lee, 1925). In every case the attempt was made to leave the parathyroids intact. In two cases, however, the parathyroids

were removed along with the thyroids, and in one of these unmistakable signs of tetany appeared. Both of these animals died in the third month of the experiment. In all of the other rats at least one parathyroid gland was left.

At the conclusion of the experiment all of the thyroidectomized animals were killed and autopsied. Except in two cases there was no evidence of regeneration of thyroid tissue. In one of these a mass of thyroid substance about one-fourth the size of one lobe had regenerated and in the other a lesser amount than this was found.

RESULTS. The significant data are presented in tables 1 and 2. It will be noted that the experimental animals showed in some, but not in all cases, a somewhat lowered activity level in the first 30-day period after thyroidectomy. Practically all of this decrease occurred in the first ten days after the removal of the glands and is ascribed to the operative trauma. Both the experimental and control rats showed a decrease in voluntary activity with age, amounting to 13 per cent in three months. The average daily activity of the male rats was only 35 per cent of that of the females. These figures agree approximately with those recorded by Hitchcock (1925).

The level of activity after thyroidectomy was somewhat surprising. One might expect that the removal of the thyroids would induce a marked lethargy. Instead, however, the total average daily activity of both the males and females for the three-month period after thyroidectomy was only 8 per cent lower than for the 30-day period immediately preceding. The average activity during this three-month period after thyroidectomy varied somewhat from that of the controls, being about 15 per cent less in the first month and only 6 per cent less in the third month after thyroidectomy. There was thus a relative increase in the activity of the experimental rats over the controls, although too slight an increase to have any positive meaning. It is clearly apparent, however, that no lethargy was produced.

DISCUSSION. That the thyroid gland bears some relationship to general well-being, as well as to the general metabolic level, is well known. The arrested development of the cretin and of the pre-pubertal myxedemic is clinical evidence that the thyroid has an important action in growth, either directly or indirectly. Experimentally, Hammett (1926) has shown for the rat, Simpson (1924) for the sheep, and Caylor and Schlotthauer (1927) for the pig, that removal of the thyroids before puberty causes a marked stunting in growth.

In the adult after growth is complete, hypothyroidism is commonly regarded as having a deleterious effect upon bodily vigor, as well as being responsible for the bodily changes of myxedema and for the decrease in the basal metabolic level. Lethargy and muscular weakness are con-

sidered common and characteristic symptoms of the condition in adults. It is probable, however, that the term lethargy is used to denote a mental dulness and sluggishness of response, rather than inability to perform muscular work. Likewise, the "muscular weakness" is seldom verified objectively, but is inferred from the patient's statement. The factors which limit the voluntary activity are probably not usually concerned with the muscular system directly, but rather with the nervous irritability of the animal. It is to be suspected that the lethargy of certain adult human myxedemics is attributable to a hypothyroid condition before puberty and a failure to develop normal nervous irritability. Liddell

TABLE 3

Showing effect of thyroid feeding on voluntary activity

RAT NUMBER	AGE AT BEGINNING OF THYROID FEEDING	AVERAGE DAILY ACTIVITY			DAILY DOSAGE THYROID SUBSTANCE	TOTAL DOSAGE THYROID SUBSTANCE
		Preliminary period 20 days	Thyroid feeding period 14 days	Post-thyroid feeding period 20 days		
	days	*revolutions*	*revolutions*	*revolutions*	*grains*	*grains*
2329 ♀	142	9464	8443	8520	$\frac{1}{8}$	1.8
2341 ♀	157	5876	6137	5962	$\frac{1}{8}$	1.8
2340 ♀	139	3116	2981	2649	$\frac{1}{4}$	3.5
2313 ♀	160	10749	10062	9782	$\frac{1}{4}$	3.5
2390 ♀	171	6218	5846	5775	$\frac{1}{2}$	7.0
2349 ♀	146	5460	5307	5568	$\frac{1}{2}$	7.0
Average....	152	6814	6463	6376		
Per cent change from preliminary period.....			−5	−6		

(1923) has shown experimentally that thyroidectomy in the sheep before puberty results in greatly diminished spontaneous activity. With this is correlated the failure of the nervous system to develop normal irritability. It should be emphasized that the rats of our experiments were thyroidectomized after sexual and bodily maturity had been attained, and were "cretins" in no sense of the word. Muscular and nervous development was complete and apparently no degeneration of these systems occurred.

The rats of these experiments, therefore, would come under the classification of adult hypothyroidism or myxedema. Such outward signs as thin hair of poor quality, dry and coarse skin, and increase in weight were noted, and support this idea. Basal metabolic rates were not determined for any of these rats before thyroidectomy, and afterwards for only two. In these two cases a marked reduction was noted, the heat production per square meter of body surface being 454 and 470 calories, respectively. In the normal rat, the average basal heat production is about 580 calories.

Lusk (1915), Benedict and Smith (1915) and Wishart (1927) have reported that previous muscular activity has a tendency to increase the basal metabolism. Athletes and persons regularly performing much muscular work were shown to have a higher basal rate than persons leading a sedentary life. Kunde and Nordlund (1927) have shown experimentally that the same is true in the dog. This raises the question of a possible reciprocal relationship between the basal metabolic level and the amount of spontaneous activity shown. In other words, after development is complete and maturity attained, will the lowering or raising of the general metabolic level alter the spontaneous activity of the animal? The lowering of the metabolic level by ablation of the thyroids, as in the experiments just described, seems to answer this question in the negative and confirmatory information is given by data from a number of other experiments and from other sources.

The effect of thyroid feeding on activity. Hoskins (1927) has shown that thyroid administration to senile rats caused no increase, but rather a decrease in voluntary activity. Other work in this laboratory has shown that thyroid feeding to young adult rats has not increased their activity. Table 3 shows the results obtained by feeding desiccated thyroid gland substance, in doses of $\frac{1}{8}$ to $\frac{1}{2}$ grain daily, to six young adult female rats for a period of two weeks. The spontaneous activity during this time is compared with that of the preceding and following twenty days. A decrease of 5 per cent occurred in the thyroid feeding period and an additional decrease of one per cent in the after-feeding period, as compared with the activity of the preliminary period. Control animals in the same time showed a slight increase in activity. The dosages employed were not large enough to produce toxic symptoms, which is important in view of the fact that any sickness is quickly manifested in a depressed activity. Wang (1927) has reported that thyroid feeding caused a considerable depression of voluntary activity. The dosages of thyroid substance which he used, however, were much greater than those Hoskins or we have employed, and the difference in results is undoubtedly due to this factor. The augmenting effect of thyroid substance on the basal metabolic rate is well known, and the conclusion is obvious that such an increase in metabolic rate as presumably occurred in these experiments, is not accompanied by a greater manifestation of spontaneous activity.

Activity and basal metabolism in the oestrous cycle. During the oestrus stage (II) of the oestrual cycle the voluntary activity of the female rat is increased from 100 to 400 per cent over the average level in the other stages (Slonaker, 1924; Durrant, 1925). If the amount of spontaneous activity manifested by the rat depends at all upon the general metabolic level, one would expect to find an increased basal metabolic rate during this stage of the cycle, characterized as it is by an enormously increased activity. At

this time, however, there is no significant change in the basal metabolic rate. More than fifty determinations of the basal metabolism during the oestrus stage in ten different rats have failed to show any constant increase or decrease as compared with the average level throughout the cycle (Lee, 1927).

TABLE 4

Showing results of basal metabolic rate determinations in male and female rats before and after removal of sex glands

RAT	AGE AT CASTRATION OR SPAYING	CALORIES/DAY/SQ. METER BODY SURFACE						PERCENT DIFFERENCE AFTER CASTRATION OR SPAYING
		Before castration or spaying	After castration or spaying				Difference	
			10 days	30 days	60 days	Average		
	days							
4367 ♂	184	580	543	552	500	532	−48	−8
4373 ♂	162	554	564	519	526	536	−18	−3
4374 ♂	184	567	529	504	521	518	−49	−9
Average...	177	567	545	525	516	529	−38	−7
Per cent change from normal			−4	−7	−9			
1265 ♀	172	604	571	525	548	548	−56	−9
4372 ♀	168	574	542	566	506	538	−36	−6
4370 ♀	168	581	537	487	513	512	−69	−12
Average...	169	586	550	526	522	532	−50	−9
Per cent change from normal			−6	−10	−11			

TABLE 5

Showing effect on basal metabolic rate of ovarian hormone injection in spayed female rats

RAT NUMBER	AGE	BASAL HEAT PRODUCTION AFTER SPAYING	BASAL HEAT PRODUCTION AFTER OVARIAN HORMONE INJECTION	TIME AFTER INJECTION
	days	*calories*	*calories*	*hours*
4370	233	512	503	39
4372	233	538	527	44

Furthermore, there is an increase in the basal metabolic rate during the last ten hours of the rest interval (stage V) and in the first six hours of the pro-oestrus stage (I). This increase amounts to approximately 12 per cent of the rate in the rest of the cycle. At this time, however, the voluntary activity is not increased, but is comparatively low. Unfortunately no activity records were obtained from the animals upon which these metabolic rate determinations were made, but the correlation of activity

and oestrus has been so well established as to render the assumption perfectly safe as to what the relative activity would have been in the various stages of the cycle.

The effect of castration or spaying. Both castration in the male (Hoskins, 1925) and spaying in the female (Wang, 1923) have been shown to have a remarkable effect on the voluntary activity of the rat, resulting in a decrease of 60 per cent or more in the male and as much as 95 per cent in the female. This decrease is not correlated with any correspondingly large decrease in the basal metabolic rate after castration or spaying. Table 4 gives the results of basal metabolic rate determinations in males and females before and after the removal of the sex glands. The technic used was the same as that described elsewhere (Lee, 1927). All determinations were made in duplicate and only those checking satisfactorily are recorded. Respiratory quotients were not determined, but a value of 0.75 was assumed. It was found that after both castration and spaying there was a variable decrease in the basal metabolic rate, amounting to about 10 per cent. Bugbee and Simond (1926) have reported a somewhat greater decrease in a castrated dog over a longer period. This decrease in the basal metabolic rate is hardly to be compared with the decrease of 60 to 90 per cent in voluntary activity.

Bugbee and Simond (1926) showed that the injection of ovarian hormone into spayed female rats causes an increase in voluntary activity comparable to that induced normally in the oestrus stage. Table 5 shows the results of basal metabolic rate determinations in two spayed female rats before and after the injection of ovarian hormone (Armour and Co. preparation). The determinations were made shortly after the cell changes in the vaginal fluid typical of oestrus had appeared. No significant change was shown.

SUMMARY

The effect of thyroidectomy on spontaneous activity was studied in eighteen adult albino rats over a period of three months. The average daily activity after the removal of the glands was essentially the same as before. No objective evidence of lethargy was obtained, although the rats were in a definitely hypothyroid condition.

Data from a number of other sources are considered bearing on the question of a possible relationship between the basal metabolic level and the amount of spontaneous activity shown by the animal.

In six young, adult female rats the feeding of thyroid gland substance caused a slight decrease in spontaneous activity.

During the oestrual cycle the changes in basal metabolic rate do not correspond with the changes in the activity level.

Castration or spaying greatly decreases the activity, but decreases the basal metabolism comparatively slightly.

The evidence is entirely against any idea that the amount of spontaneous activity manifested by an animal is dependent upon the basal metabolic level. Neither does the basal metabolic level show any close variation with the amount of spontaneous activity manifested.

BIBLIOGRAPHY

BENEDICT AND SMITH. 1915. Journ. Biol. Chem., xx, 243.
BUGBEE AND SIMOND. 1926. Endocrinol., x, 349; This Journal, lxxv, 542.
CAYLOR AND SCHLOTTHAUER. 1927. This Journal, lxxix, 141.
DURRANT. 1924. This Journal, lxx, 344.
1925. Endocrinol., ix, 221.
HAMMETT. 1926. This Journal, lxxvi, 69.
HITCHCOCK. 1925. This Journal, lxxv, 205.
HOSKINS. 1925. This Journal, lxxii, 324.
1927. Endocrinol., xi, 136.
KUNDE AND NORDLUND. 1927. This Journal, lxxx, 681.
LEE. 1925. Endocrinol., ix, 410.
1927. This Journal, lxxxi, 492.
LIDDELL. 1923. Quart. Journ. Exper. Physiol., xiii, 191.
LUSK. 1915. Journ. Biol. Chem., xx, 555.
SIMPSON. 1924. Quart. Journ. Exper. Physiol., xiv, 161.
SLONAKER. 1924. This Journal, lxviii, 294.
WANG. 1923. Comp. Psychol. Monographs, Series 2, no. 6.
1927. Bull. Johns Hopkins Hosp., xl, 304.
WISHART. 1927. Quart. Journ. Med., xx, 199.

EFFECTS OF THYMUS, MUSCLE AND PITUITARY EXTRACTS ON NORMAL AND THYRO-PARATHYROIDECTOMIZED DOGS

E. LARSON AND N. F. FISHER

From the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois, Chicago, Ill.

Received for publication December 5, 1927

After our (Fisher and Larson, 1925) successful attempts in controlling tetania parathyreopriva with extracts of beef parathyroids, we became interested in investigating the effects of similar extracts prepared from other tissues. Possibly there might be a parathyroid-like principle in other tissues than in the parathyroids analogous to the insulin-like substance foreign to the pancreas. The thymus gland was first investigated because of its similar embryological origin. Ott and Scott (1909) claimed that parathyroid tetany was not influenced by extracts prepared by triturating dried thymus gland with saline solution. These investigators (Ott and Scott, 1908, 1909) also reported that tetany could be alleviated by pituitary extract.

Procedure. A series of dogs were thyro-parathyroidectomized by removing the thyroid capsule and the contained thyroid and parathyroid glands. The diet consisted of hashed meat and dog biscuit. All of the extracts were administered subcutaneously. The calcium determinations were made by the Clark and Collip method (1925), the blood sugar as described by Folin-Wu, the inorganic phosphorus by Tisdall's procedure (1922) and the non-protein nitrogen by the method of Koch-McMeekin (1924).

Methods of preparing the extracts. Extract Th-1. This was prepared from the fresh thymus glands of calves by our method for preparing parathyroid extract A, 1 cc. represented 3 grams of fresh tissue.

Extract Th-2. The insulin method as used by one of us (Fisher, 1923–24) was applied to the calf thymus. Before extraction the glands were freed from fat and connective tissue, ground, placed in a ball-mill with the acid alcohol and ground for six hours, 1 cc. represented 10 grams of the thymus gland.

Extract Mu-1. This was prepared from the fresh muscle tissue of a calf by the method applied to extract Th-1, 1 cc. = 3 grams.

Extract Mu-2. To an equal volume of extract Mu-1, aluminium cream as

prepared by the method of Marshall and Welker (1913) was added. After thoroughly mixing, this material was filtered and concentrated so that 1 cc. was equivalent to 2 grams of tissue.

Extract P. The standard pituitary infundibular powder prepared according to the directions of Smith and McClosky (1924) was extracted with boiling water, filtered and diluted so that 1 cc. = 5 mgm. of the standard powder.

The extracts were always kept in an icebox and 0.1 per cent tricresol was added as preservative.

In the series of 34 dogs which were thyro-parathyroidectomized the effects of the extracts were as carefully studied as possible. During the first part of the investigation some of the animals were injected with the thymus extract before tetany developed. The other dogs were injected only when tetany was evident, in order to contrast the curative effect with its power as a preventive.

The following protocols were selected as typical of the effect of the extracts.

Dog W. Female. Weight 10 kilos

October 23, 1925. 2:00 p.m. Thyro-parathyroidectomized
October 24. Condition good. Ate meat
October 25. Condition good. Ate meat
7:30 p.m. Violent attack of tetany; 10 cc. of extract Th-1 injected
9:00 p.m. Mild tetanic symptoms; 5 cc. extract Th-1
October 26. 2 p.m. Condition excellent
5 p.m. Severe attack of tetany; 12 cc. extract Th-1
October 27. 10:00 a.m. Condition very good
9:30 p.m. Hyperpnea, etc., 12 cc. extract Th-1
October 28. 10:30 a.m. Signs of tetany; 12 cc. extract Th-1
1:00 p.m. Condition good
10:30 p.m. Impending tetany; 12 cc. extract Th-1
October 19. Ate some meat
October 30. Ate some meat. Tetany; 12 cc. extract Th-1
October 31. 11:00 a.m. Impending tetany; 12 cc. extract Th-1
November 1. 12:30 p.m. Ate some meat; 12 cc. extract Th-1
2:00 p.m. Tetany; 12 cc. extract Th-1
November 2. Some stiffness of hind legs; 8 cc. extract Th-1
November 3. 1:00 p.m. Signs of tetany; 10 cc. extract of Th-1
Nov. 4 and 5. No tetany but did not eat
November 6. Fed 1 egg and 50 grams of sucrose in 200 cc. of water by stomach tube
November 7. Condition fair
November 8. Dead. Emphysema of right lung and complete red hepatization of middle lobe. Pneumonia

Dog A-16. Female. Weight 11 kilos.

April 24, 1926. 9:15 a.m. Thyro-parathyroidectomized
April 25. Condition good
April 26. a.m. Ate meat
3:00 p.m. Signs of tetany; 5 cc. extract P
10:30 p.m. Impending tetany; 5 cc. extract P

April 27. Condition excellent
4:30 p.m. Calcium of blood serum 4.31 mgm. per 100 cc.
April 28. Did not eat much. Signs of tetany
3:30 p.m. Signs of tetany; 5 cc. extract P
8:00 p.m. Signs of tetany; 5 cc. extract P
April 29. a.m. In excellent condition
2:00 p.m. Impending tetany; 5 cc. extract P
4:30 p.m. No symptoms of tetany. Blood serum calcium 3.97 mgm. per 100 cc.
April 30. Very much depressed
May 1. Very much depressed
May 2. 1:00 a.m. Very much depressed
11:30 a.m. Dog dead. Dog evidently did not die of tetany as the legs were not extended nor the head thrown back. Neither was there any evidence of salivation. Only findings at autopsy were two healed infarcts on the spleen

Average length of life after thyro-parathyroidectomy of the groups with various treatments

METHOD OF CONTROL OF THE TETANY	NUMBER OF DOGS IN THE GROUP	AVERAGE LENGTH OF LIFE IN DAYS
Extract Th-1 before tetany developed	6	5.3
Extract Th-1 after tetany developed	6	7.8
Extract Th-2 after tetany developed	7	5.4
Extract Mu-1 after tetany developed	4*	5.2
Extract Mu-2 after tetany developed	4	7.5
Extract P-1 before tetany developed	3	5.6
Extract P-1 after development of tetany	1	8.0

* One dog (A-18) which lived 117 days not included because of probable accessory parathyroid.

The two following tables were selected as being typical of the effect of these extracts on the chemical constituents of the blood. The same analyses were made after the injections of the other extracts. Since their results were nearly identical they are not included in this paper.

Effects of extracts on the chemical constituents of the blood

TIME	EXTRACT ADMINISTERED	NON-PROTEIN NITROGEN PER 100 CC. OF BLOOD	SUGAR PER 100 CC. OF BLOOD	CALCIUM PER 100 CC. OF SERUM	INORGANIC PHOSPHORUS PER 100 CC. OF SERUM	REMARKS
Dog N. Young male mongrel. Weight, 27 kilos						
0	8 cc. extract Th-1	41.2	97.5	10.67	4.37	
11	every hour for 9	46.1	109.2	10.34	5.28	
24	hours	36.1	116.2	11.50		
31		37.2	126.4	10.16	4.45	
48		39.7	107.8	10.98	4.02	

Dog O. Male. Weight, 15 kilos

0	10 cc. of extract P	31.52	85.4	9.77	4.62	Vomited some dur-
11	every hour for 9	29.54	103.0	9.04	5.73	ing the 5th and
24	hours	33.50	109.8	9.10	4.78	6th hour
31		26.48	106.8	9.10	4.40	
48		38.10	99.6	10.12	5.86	

Discussion. From the data which we have at hand it is evident that in all the tissues which were extracted there is some protective substance present which has a beneficial effect on parathyroid tetany. But we do not, however, believe that it is a very powerful protective substance. This substance which has an alleviating influence on the acute attacks of tetania parathyreopriva seems to be present in greater amounts in the thymus than in the other tissues examined.

In the dogs which were injected with thymus extract before tetany developed in an effort to prevent tetany the average length of life was 5.3 days. This we find to be considerably above that which the average parathyroidectomized dog will live without any treatment. Dog C, which lived 14 days and had definite signs of tetany at least three times, did not seem to care to eat during the last few days of his life. With dog E there was an alleviation of one decided attack of tetany which occurred on the second day after the operation. Though the animal lived for five days more without an attack, it was not in good condition. The day after the attack the dog still showed muscle tremors in the temporal region and there was spasticity of the leg muscles so that the animal walked on the back of the foot.

When the thymus extract was injected only after tetany had developed, or was impending, the average length of life was 7.8 days. With dog W, eleven attacks of tetany were relieved but the dog died on the fifteenth day from pneumonia. In this series dog V is interesting because of the recovery from one attack when extract was injected. This animal did not eat during the last ten days of his life and had to be fed by means of a stomach tube. Marked spasticity of the muscles was readily elicited by touching the animal.

Since these extracts contained a fairly large amount of protein material it was thought that extract Th-2 which was prepared by a modification of an insulin procedure might be as effective and not show any effects which might be attributed to the protein present. The average life in this group was 5.4 days. Two of the cases, dog A-8 and dog A-11, are interesting because the animals showed a hypocalcemia even after relief from tetany. The blood serum calcium values for dog A-8 were 5.76 and 4.9 mgm. per 100 cc. The latter of these two cases deserves especial attention because this animal had an attack of tetany which was of a convulsive type on the

sixteenth day after operation. The calcium determinations had been 5.61, 5.4 and 3.98 mgm. in the period preceding the attack. Before the attack the animal had had muscle tremors for several days but was not injected because no other symptoms were present. On the day following the attack, the calcium value was 4.98. The animal was found dead on the second day after the attack of tetany. The cause of death was undetermined. We feel that an animal may not show an attack of tetany even if the calcium level is below the tetany level (7 mgm.) if the fall is gradual. This has been pointed out previously by Salvesen (1923) and also by ourselves (1925).

These results with thymus extract which show that it has an alleviating influence in the acute tetany due to parathyroidectomy are very interesting because Uhlenhuth (1917, 1919a and 1919b) in a series of experiments with salamanders which were fed thymus concluded that thymus, and the parathyroid glands are antagonistic. The symptoms produced by the feeding of thymus are not comparable to acute parathyroid tetany in dogs. Furthermore Hoskins (1918) has questioned the effects produced by feeding of thymus because it has not been shown that thymus alone is an adequate diet. Cameron and Carmichael (1925) fed thymus glands to rats in an effort to increase the incidence of tetany in rats which were fed thyroid gland. Their results do not support Uhlenhuth's hypothesis. Following intraperitoneal injections of thymus extract Olkon (1918) produced muscular spasms and convulsions, but Downs and Eddy (1920) reported contradictory results when thymus extract was injected subcutaneously into young rabbits. The specific toxin which Olkon ascribed to thymus is open to question because when desiccated muscle tissue was injected somewhat similar results were produced. In the guinea pig Honeyman (1919) has shown that thymectomy does not modify the tetany syndrome produced by extirpation of the parathyroids. From the results of these investigators, as well as ours, it appears unlikely that the thymus contains any substance capable of inducing tetany.

The temporary relief of parathyroid tetany by the injection of pituitary extract has been reported by Ott and Scott (1908, 1909) but very little actual data were presented. From their results they concluded that the infundibular lobe extract is the active constituent in the alleviation of the symptoms of tetany. Our results with pituitary extract were not conclusive. We feel that the average length of life was increased but the effect of the extract was only temporary. The results with dog A-16 are similar to those which received thymus extract. The dogs were relieved of tetany but the calcium of the blood serum was not increased and the animals eventually died. The low calcium values of the blood of this dog are significant. According to the work of Bell and Hick (1909) pituitary extract should have a powerful influence on tetany because according to

these investigators the calcium content of the blood is increased by injections of pituitary extract.

The results with muscle extracts were not as good as with the other extracts, the average length of life of the two groups treated with muscle extract was 5.2 and 7.5 days. One of the dogs in this group A-24 which was injected with extract Mu-2 on two occasions showed calcium values of 6.04 and 5.54 at times when no symptoms of tetany were present. The attacks of dog A-21 were controlled but the animal did not seem to care to eat, and finally died in an attack of tetany when no treatment was used.

All of these extracts seem to have some ameliorative action on the acute attacks of parathyroid tetany. But they do not have the power of increasing the calcium concentration of the blood serum of either normal or thyro-parathyroidectomized dogs. There is a slight indication that thymus extract may cause a slight increase in calcium but as the amounts injected in order to study the effect on the chemical constituents of the blood were much larger than those injected for the relief of tetany, no significance is attached to this slight increase. The method used in preparing the extract was exactly the same as used for preparing a potent parathyroid extract from the ox parathyroids. The alleviating action of these extracts in tetany can not be explained on a calcium basis, that is, they do not control tetany because of their action in increasing the calcium of the blood serum. However, Leites (1924) claims that the calcium of the blood decreases after thymectomy in the rabbit. If such is the case then an extract of this gland might have the power to increase the calcium of the blood. The effect of these extracts seem to be very temporary as the animal eventually dies from conditions induced by the hypocalcemia even if the extract does relieve the acute attacks of tetany.

The results produced by the pituitary and muscle extracts are similar to those produced by the thymus extract. The results of several other investigators are at variance with our findings. Bell and Hick (1909) reported that pituitary extract increased the calcium content of the blood but their findings can be disregarded because of the inaccuracies of the method which they used. Recently Davies, Dickens and Dodds (1926) reported that pituitrin increases the calcium content of the blood.

The action of these extracts especially the thymus and muscle was first thought to be due to the protein content, since the protein content of the thymus extract (150 mgm. per 100 cc.) was three times as great as that of our parathyroid extract. It was thought the depression and anorexia in some of the animals was due to the fever caused by the injections but no increase in temperature of two normal animals was noted after injections of larger amounts than were used for the relief of tetany. The effect on tetany with the extracts which had at least all of the higher proteins (proteoses and the higher polypeptides) removed by treatment with alumin-

ium cream was not different in any respect from those which had not been treated. The depression and anorexia has also been noted by Boyd, Austin and Ducey (1926) when they attempted to control parathyroid tetany by means of oral administration of ammonium chloride. The variations in the chemical constituents of the blood produced when these extracts were injected into normal animals were insignificant. There was no decided increase of the calcium in any case, but with the thymus extract Th-1 there was a slight indication in both cases. One of these dogs was depressed and the blood was concentrated as well. But we cannot explain the action of these extracts in alleviating the acute attacks of parathyroid tetany on the basis of their effect on the calcium of the blood. The exact mechanism is not understood. The animals were not normal after relief by this extract but were in a tetanoid condition, and the spastic state of the skeletal musculature which is so prominent in tetany could readily be induced by excitement of the animal. The animals seem to be able to adjust themselves to their lowered calcium of the blood if the fall is gradual. The reason for the absence of the attacks during the period when the amount of calcium is below the tetany level is that the body has adjusted itself to this new low level.

CONCLUSIONS

1. Extracts of calf thymus and muscle prepared by the same methods used for preparing potent parathyroid extracts do have a slight ameliorative action on acute parathyroid tetany.

2. These extracts as well as pituitary extracts do not cause sufficient changes in the calcium of the blood to account for their ameliorative action.

3. We have been unable to demonstrate any parathyroid-like principle in the extracts which we prepared.

We wish to express our thanks to Dr. A. J. Carlson for his revision of the manuscript.

BIBLIOGRAPHY

BELL AND HICK. 1909. Brit. Med. Journ., 1, 517.
BOYD, AUSTIN AND DUCEY. 1926. This Journal, lxxvii, 225.
CAMERON AND CARMICHAEL. 1925. Trans. Royal Soc. Canada. Sec. v, xix, 53.
CLARK AND COLLIP. 1925. Journ. Biol. Chem., lxiii, 460.
DAVIES, DICKENS AND DODDS. 1926. Biochem. Journ., xx, 695.
DOWNS AND EDDY. 1920. Endocrinol., iv, 420.
FISHER AND LARSON. 1925. This Journal, lxxv, 93.
HONEYMAN. 1919. Journ. Physiol., liii, 207.
HOSKINS. 1918. Endocrinol., ii, 241.
KOCH AND MCMEEKIN. 1924. Journ. Amer. Chem. Soc., xlvi, 2066.
OLKON. 1918. Arch. Int. Med., xxii, 815.
OTT AND SCOTT. 1908. N. Y. Med. Journ., lxxxiii, 118; xc, 359.

LEITES. 1924. Biochem. Zeitschr., cl, 183.
MARSHALL AND WELKER. 1913. Journ. Amer. Chem. Soc., xxxv, 820.
SALVESEN. 1923a. Proc. Exper. Biol. and Med., xx, 204.
1923b. Journ. Biol. Chem., lvi, 445.
SMITH AND MCCLOSKY. 1924. Hygienic Lab. Bull., no. 138.
TISDALL. 1922. Journ. Biol. Chem., l, 327.
UHLENHUTH. 1917. Proc. Exper. Biol. and Med., xv, 37.
1919a. Proc. Exper. Biol. and Med., xvii, 70.
1919b. Endocrinol., iii, 285.

SIMULTANEOUS DETERMINATION OF THE PULMONARY AND SYSTEMIC CIRCULATION TIMES IN MAN AND OF A FIGURE RELATED TO THE CARDIAC OUTPUT

W. F. HAMILTON, JOHN WALKER MOORE, J. M. KINSMAN AND R. G. SPURLING

From the Departments of Medicine, Surgery and the Department of Physiology and Pharmacology, University of Louisville

Received for publication December 9, 1927

It has been known for nearly a hundred years (1) that it is possible to determine the time of transport of a particle of blood from one part of the cardiovascular system to another, by injecting an easily detected substance into the cardiovascular bed at one point and determining its time of appearance at another point. Thirty years ago Stewart (2) showed that it was possible to estimate the quantity of blood put out by the heart in animals, from the dilution of a known amount of injected substance by the blood which passes through the heart during a known period of time.

Interest in this approach to the physiology of the circulation has lagged until the recent ingenious investigations of Blumgart, who has described (3) an elaborate method for determining the pulmonary circulation time in man and animals.

The experiments described below aim at applying the methods of the earlier workers in such a manner as to determine, as a result of one and the same experiment: 1, the pulmonary circulation time; 2, the systemic circulation time, and 3, a figure which is closely related to the cardiac output in experimental animals or in man.

The method, in brief, is to inject an easily detectable substance into a vein and time its appearance and reappearance in blood from the radial artery in man and the right and left ventricles in dogs.

Apparatus and procedure. (See fig. 1.) Around the circumference, at the top of a Harvard kymograph carrying the usual smoked paper, were placed small, thin-walled glass tubes, each about 6 mm. inside diameter and 3 cm. long, drawn to a blunt point below. They were held in place by a rubber band; they were further kept from dropping by a fairly heavy rubber tube below them and upon which they rested. This tube also served the purpose of keeping blood from running down upon the paper and signal points below. The latter consisted of 1, a time marker, recording seconds; 2, a signal marker recording the beginning and end of the

injection, and 3, a pulse recorder, either a key and signal magnet, worked by hand or a tambour with impulse transmitted from a cup placed over an artery.

The speed of the drum was regulated so that each tube took about one or two seconds, in different experiments, to pass a given point.

In some of the experiments one drop of isotonic potassium oxalate solution was placed in each tube. It was found that this usually prevented clotting and that hemolysis was avoided by removing standing drops of blood from the side of the tube (hemolyzed blood interferes with colorimetric determinations). Blood usually settled sufficiently after standing for a time, so that the plasma could be used without further throwing down of the cells; but if this became necessary the tubes could be conveniently placed in a centrifuge tube stuffed with cotton, and the cells quickly packed. In other experiments no oxalate was used, the blood being allowed to clot and serum obtained by centrifugalization.

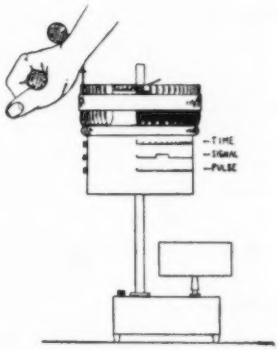


Fig. 1. Apparatus for collecting blood from the radial artery to determine the circulation time.

Two hundred and fifty to 700 mgm. of phenol-tetraiod-phthalein sodium[1] were dissolved in 1 to 1½ cc. of physiological saline (saline was found to be a more satisfactory solvent than distilled water, for the substance precipitated in the latter). The reason for selecting this substance is that it has been shown (4) that it is secreted slowly, and that it is possible to do quantitative determinations in the blood plasma. Our own experiments indicate that the substance is not absorbed appreciably by the cells.

In the human experiments the subject sat on a stool in such a position that the palmar surface of the wrist was directly above the collecting tubes (fig. 1). The drum was pushed aside, the needle (20 gauge, 4 cm. long with syringe adapter removed and a brass collar soldered around the middle) was inserted in a perpendicular direction into the radial artery, which should be novocainized. Once a free flow of blood was obtained the drum was placed in position so that the blood fell into a tube, marked by a stationary pointer, directly above the signal.

The drum was then started. When it was well under way the injection, (300 to 700 mgm. in 1 to 1½ cc. of saline) was made as rapidly as possible, the beginning and end being signaled (1 to 1.5 seconds). Time and pulse were recorded.

[1]Manufactured by Mallinckrodt Chemical Works, St. Louis, Mo.

In the dog experiments the same procedure was followed except that 200 to 250 mgm. of the substance were injected into the (exposed, anchored)

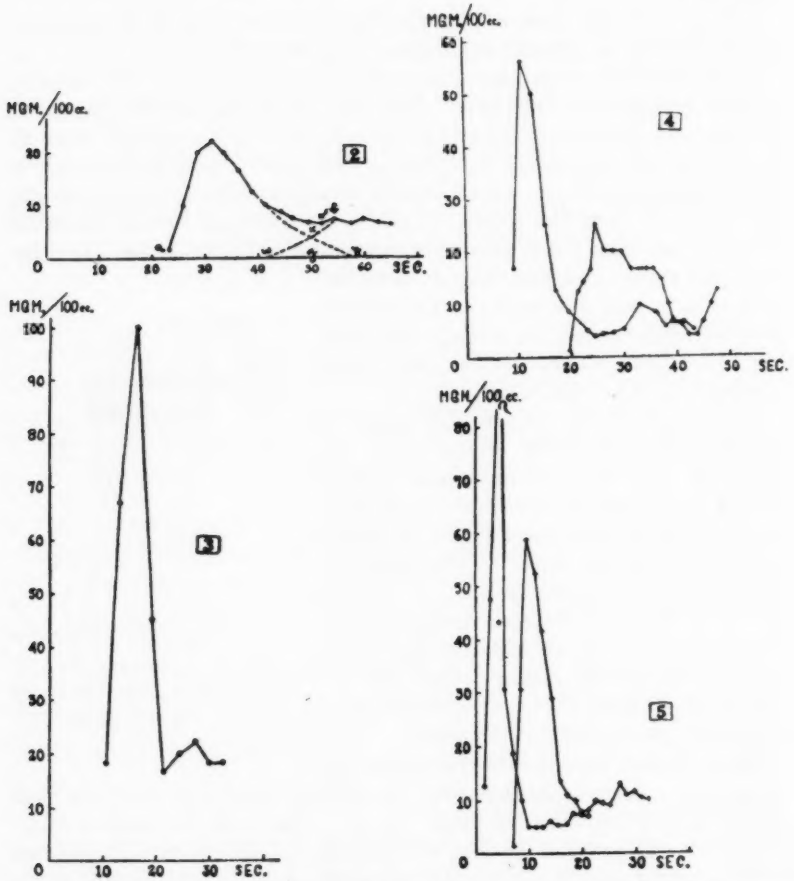


Fig. 2. Curve of concentration of injected substance in blood from the radial artery of a man weighing 85 kgm., pulse 72; 300 mgm. phenol-tetraiod-phthalein Sodium injected into jugular; *b* markes the point when recirculated blood becomes evident.

Fig. 3. Similar to figure 2. Man 75 kgm., pulse 124; 700 mgm. injected.

Fig. 4. Curve of concentration of injected substance in blood from the right heart ●, and from the left heart +, of a morphinized dog weighing 30 kgm., heart rate 60; 250 mgm. injected.

Fig. 5. Similar to figure 4. Same dog undrugged. Heart rate very rapid; 250 mgm. injected.

jugular vein and simultaneous samples were collected from the right and left ventricles. The needles used were 13 gauge for the right ventricle and 15 gauge for the left, each 15 cm. long. The dog was suspended above the drums in a jacket with a hole over the heart. The cardiac punctures were made under novocaine, and in some experiments 100 mgm. of heparin were injected to slow clotting.

The remainder of the procedure followed the human experiments.

As a result of this procedure we had consecutive samples of the blood from the radial artery in man and from the right and left ventricles in dogs. It was possible to estimate the time of appearance and the concentration of the dye in each series.

To make these estimations we measured in a capillary pipette, the same quantity (25 to 50 cmm.) of plasma or serum from each tube and added it to 1 cc. of 1 per cent alkali in water. The concentration of the substance in mgm. per 100 cc. was estimated colorimetrically against standards made by diluting the substance in known quantities of the blood of the experimental subject. These small quantities can be handled very satisfactorily in the Bausch and Lomb microcolorimeter.

EXPERIMENTAL RESULTS. *Human experiments.* In the experiment illustrated in figure 2, 350 mgm. of the compound were injected into the jugular vein of a large individual (about 85 kgm.) His cardiovascular system was apparently normal; pulse 72. He was not excited or worried over the experimental procedures.

The injected substance first appeared in the radial stream 23 seconds after injection. The concentration reached a peak at 31.5 seconds and then declined steadily until 52 to 54 seconds. At this point there was a secondary rise. This secondary rise occurred in all curves plotted from experiments performed under the above conditions, and seems to be due to some of the injected substance which has passed through the left heart twice.

If we let a equal the time it takes the most rapidly flowing blood to get through the right heart, b through the pulmonary circuit, c through the left heart, d through the shortest systemic circuit, x from jugular to heart, and y from heart to radial artery:

$$\text{Then, } x + a + b + c + y = 23 \text{ seconds}$$

Allowing one second each for x, a, c and y, from evidence adduced later in this paper and by Blumgart (3)

$$b = 19 \text{ seconds, i.e., the pulmonary circulation time.}$$

If our interpretation of the secondary rise is correct, the 54 seconds between the time of injection and this rise would be made up of:

$$x + 2\,(a + b + c) + d + y = 54 \text{ seconds}$$

substituting and solving for d

d = 10 seconds, i.e., the shortest systemic circulation time.

In the experiment illustrated in figure 3, the circulation was obviously much more brisk. The heart rate was 124 per minute, as the subject was somewhat excited by the procedure.

When the results of the experiment are calculated for d and b as in the previous experiment:

b = 6.7 seconds—the pulmonary circulation time.
d = 4.8 seconds—the shortest systemic circulation time.

Experiments on dogs in which we tried to get at the relation between the greater and lesser circulation times more directly, gave results which confirmed the calculations from the human experiments.

Thus, in the experiment illustrated in figure 5, and in several other incomplete experiments the injected substance leaves the right ventricle during the first 1 to 2.5 seconds ($x + a$) giving evidence that the circulation time between the jugular vein and the pulmonary artery is very small. The delay of 9 seconds in figure 4, may be an actual picture of conditions in the circulation under morphine, but we are inclined to regard it from the other experimental data as an error and to assume that the injected substance enters the heart very much more quickly.

The time that it takes the substance to get from the right heart to the left heart is 5.5 seconds (fig. 5) in the normal dog and 10.5 (fig. 4) in the same animal under morphine.

The time that it takes for the blood to take its quickest route from the left heart back to the right heart is approximately 12 seconds under morphine and 6 seconds without the drug. These time values are concordant, whether they are calculated from the arterial stream alone or directly observed from the relationships of the time values in the right and left heart.

Calculation of the cardiac output is a tentative procedure; the results are illustrative and in no sense final. It is to be emphasized, however that the injection method of calculating the cardiac output is more accurate when carried out according to the procedure outlined below, than when following the older procedures. The older workers had no means of being sure that blood had not re-circulated before they stopped sampling and hence were not certain that they had not measured some of the injected substance twice. The new procedure indicates clearly the time when recirculation begins and hence makes the estimation more definite than the earlier procedures. This and its applicability to man leads us to hope that when the method is further developed and its accuracy checked, it will prove useful.

In principle, the object of the procedure is to determine the amount to which the injected substance has been diluted by the blood passing through the heart and lungs during the time of the first circulation and to calculate the cardiac output per minute from the amount of blood which has diluted the known amount of injected substance over a known time.

The average concentration of the injected substance in experiment 1, during the time the first circulation was passing the radial artery was 12.7 mgm. per 100 cc. This was determined colorimetrically by mixing the samples from the beginning of the curve to *b* (fig. 2).

If all 300 mgm. were to be diluted to this concentration, it would require 2.36 L. of blood. Since this quantity of blood was mixed with the injected substance during 27 seconds the rate of blood flow comes out at 5.22 L. per minute.

A similar calculation for experiment 2 gives almost the same figures, *i.e.*, 5.2 L per minute. Although the heart was beating nearly twice as fast as in the first experiment the cardiac output did not show a corresponding increase (5).

The cardiac output as calculated from the dilution of the substance in the dog's blood can be based upon the average concentration in either the right heart or in the left heart. The two calculations act as independent checks upon each other. It is to be emphasized that these figures are tentative and must be checked up by later work in which the direct Fick method is used as a control.

The figures, such as they are, in the morphinized dog, stand 4.1 L. per minute from the right heart and 4.2 from the left.

The same dog undrugged gave 3.0 L per minute from the right heart and 2.7 from the left. We suppose that the output from the two sides of the heart is approximately the same in each experiment and that the differences (2.5 per cent and 10 per cent) are due to various errors. The fact that the differences are relatively small indicates that the various errors have a tendency to cancel each other. It remains, of course, to look for a possible systematic error that affects both sets of data alike.

This method of calculating the cardiac output might be objected to on the ground that some of the injected substance remains in the lungs and is not included in the calculation, rendering the final figure too high.

Such blood would be represented by the hypothetical curve *xyz* (fig. 2). It may be possible when the results of further experiments are interpreted to calculate the dye represented in this hidden portion of the curve. But at present, in experiments in which x' is low and consequently the area of *xyz* small, it is sufficient to ignore that portion of the lost substance that is not canceled by *wxz*, i.e., the substance going through on its second circulation but in concentration too weak to show on the curve.

In some curves, notably those taken from decompensated cardiac pa-

tients, where x' is high, indications are that the "cardiac output figure" is vitiated by the large amount of injected substance lost in the lungs, represented by a large xyz.

SUMMARY

A method is described which makes possible in man and animals simultaneous determination of: 1, the pulmonary circulation time; 2, the systemic circulation time, and 3, a figure derived from the dilution of an injected substance by the blood—a figure which is probably very closely related to the cardiac output.

BIBLIOGRAPHY

(1) HERING, E. Zeitschr. f. Physiol., 1829, iii, 85 (quoted from Schafer's Text-book of physiology).
(2) STEWART, G. N. Journ. Physiol., 1897, xxii, 159 (quoted from G. N. STEWART, This Journal, 1921, lvii, 27. This paper reviews the later literature on calculating the cardiac output by injection methods).
(3) BLUMGART, H. L. AND S. WEISS. Journ. Clin. Invest., iv, 399, 1927.
(4) GRAHAM, E. A., W. H. COLE, G. H. COPHER AND S. MOORE. Journ. Amer. Med. Assoc., 1926, lxxxvi, 476.
(5) MARSHALL, E. K., JR. This Journal, 1926, lxxvii, 459.

XVIII. THE DEPRESSOR EFFECT OF EPINEPHRIN IN DECEREBRATED ANIMALS

CHARLES M. GRUBER

From the Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo.

Received for publication January 9, 1928

In 1895 Oliver and Schäfer (1) demonstrated that adrenal extract has a pressor action upon the vasomotor system in both frogs and dogs. In the dogs they plethysmographed several organs simultaneously. These did not of necessity respond in the same manner, at times one contracted and the others expanded and vice versa, and sometimes all the organs under observation expanded. This increase they attributed to a passive expansion due to swelling of the large blood vessels in the limbs. Their results were confirmed one year later by Szymonowicz (2).

It has been shown that epinephrin injected into animals (cats and dogs) in small doses (0.1 to 0.5 cc. of a 1:100,000 solution) prcduces a fall in blood pressure. This fall Cannon and Lyman (3) say results not only from a lessened peripheral resistance due to vasodilatation in the cutaneous system but also to dilatation in the splanchnic vessels. Plethysmographic records taken simultaneously with blood pressure records showed that the limb volume increases as the blood pressure falls. Dale (4) came to the same conclusion as Cannon and Lyman. He advanced the theory that small amounts of epinephrin stimulate vasodilator endings while large quantities bring the vasoconstrictors into play.

Hartman (5) does not share absolutely in this belief, but maintains that epinephrin in small doses is selective in its action. He found that if the limb vessels were clamped epinephrin produced a rise in blood pressure, and if the vessels of the splanchnic area were clamped and the limb vessels left intact epinephrin produced a fall in blood pressure. He says that toneless relaxed muscles probably do not relax further by injection of epinephrin. Later Hartman and McPhedran (6) and Hartman and Fraser (7) advanced the theory that the vasodilator action of epinephrin in muscle vessels does not depend upon the tonicity of the vessel wall but upon a center in the central nervous system elsewhere than in the cerebral hemispheres. They believed they obtained dilatation in the perfused limbs of dogs upon injecting epinephrin into the circulation of the animal when the nervous connections were intact. Upon further experimenta-

tion Hartman and his co-workers (8) ultimately concluded that epinephrin acts on both "gangliar" and "peripheral" mechanisms in producing dilatation of the hind limb of the cat and of the dog.

Hoskins, Gunning and Berry (9) pointed out that epinephrin injected intravenously in small doses dilates the muscle vessels and constricts the cutaneous ones. Gruber (10) was unable to obtain vasodilatation in muscle vessels the nerves of which had just been cut. His results were confirmed by Hartman and Fraser (7). Nor was he able to obtain dilatation in active muscles whose nerves were cut and stimulated at a rate favorable to dilatation.

Working independently, Gruber (11) and Dale and Richards (12) simultaneously came to the same conclusion, i.e., epinephrin produces its fall in blood pressure and dilatation of the muscle vessels primarily by its peripheral action on the blood vessels, independent of the integrity of any nervous connection—and secondarily by reflexly depressing the vasoconstrictor mechanism in large doses. These results have been corroborated by Pearlman and Vincent (13) in their studies on the function of the chromophil tissues.

Recently McDonald and Schlapp (14) published a report to the effect that the fall in blood pressure produced by small doses of "adrenalin" occurs only during anesthesia by ether or urethane and is not observed in animals decerebrated and the ether discontinued for some time before the injection.

Inasmuch as the author recalled that he had used epinephrin on some decerebrated animals in his previous work (15) but had failed to note any results other than the usual action of the drug this work was done to confirm or deny if possible the findings of McDonald and Schlapp.

Method. Eight cats, decerebrated under ether anesthesia, were used. All the operative work except the insertion of the cannula into the femoral vein was completed before the ether anesthesia was discontinued. The animals were tracheotomized.

The blood pressure was registered by a mercury manometer from a cannula in the carotid artery. A time marker was placed at the atmospheric pressure level of the manometer.

After the completion of the operative work the ether was discontinued and a period of from thirty minutes to three and one-half hours was allowed before the action of epinephrin was tested.

Adrenalin chloride, 0.1 to 1 cc. of a 1:100,000 solution, was injected into a cannula placed in the external jugular vein. A paraffined glass cannula of small bore was placed in the femoral vein, all the branches to which were tied off except a small vein which came from the adductor femoris muscle. The drops of blood flowing from it were recorded on the smoked drum surface by receiving and recording tambours.

RESULTS. In the eight decerebrated cats in which epinephrin was tested the results from the first injection differed in no respect from those found in anesthetized animals. In six of these eight cases the first injection of epinephrin was followed by a pronounced fall in blood pressure. In the other two the original blood pressure was below the critical level (70 to 90 mm. Hg) pointed out by Cannon and Lyman (3) and therefore no fall resulted. In one of the above six animals 0.3 cc. of adrenalin chloride 1:100,000 was injected thirty minutes after the ether anesthetic was discontinued. A fall of 27 per cent in blood pressure resulted. Four other injections were made each of which was followed by a fall in blood pressure the last being made three hours after ether had been discontinued. Usually after the second but occasionally not until after the third injection, a rise in blood pressure occurs. Ordinarily subsequent injections result either in elevations in blood pressure or in marked oscillations in blood pressure. In two of the five remaining animals, in which a definite fall in blood pressure was noted, the time between the end of the operation and the beginning of the epinephrin experimentation was one and one-half hours, in two others two hours and in one, three and one-half hours. In four of these the second injection was followed by a rise in blood pressure, two being only slight temporary rises preceding falls. In the other animal such changes were noted only after the third injection.

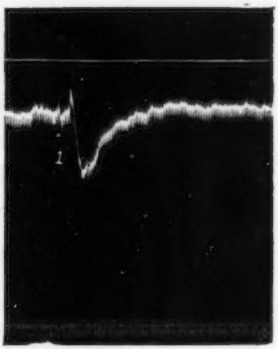


Fig. 1. Male cat, decerebrated and ether discontinued 30 minutes before injection of epinephrin. Upper record, blood pressure with mercury manometer. Lower record, time interval in 5 seconds and zero blood pressure. *1*, 0.3 cc. adrenalin chloride 1:100,000.

Figure 1 was written by a decerebrated male cat which had no ether during the thirty minutes just preceding the injection of adrenalin chloride (0.3 cc. 1:100,000) at *1*. As a result of the injection the blood pressure fell from 110 to 80 mm. of mercury.

The animal from which figure 2 was made, was decerebrated and the anesthetic discontinued one and one-half hours before the injection of the drug. In this case the rate of blood flow from the skeletal muscle as well as the blood pressure is illustrated. In both instances 0.2 cc. of a 1:100,000 solution of adrenalin chloride was injected. Following the first injection, *1*, the blood pressure after a temporary rise fell from 105 to 95 mm. of mercury. The second injection at *2* shows no noticeable effect upon the blood pressure. All subsequent injections caused elevations in blood pressure. In this instance both injections shown in the record caused

an increased blood flow in the skeletal muscle. This increase amounted to 23 drops in 30 seconds in *1* and 18 drops per 30 seconds in *2*.

In some animals marked oscillations in blood pressure occur following the injection of epinephrin. These changes are frequently noted as the result of similar injections in anesthetized animals. In the latter animals

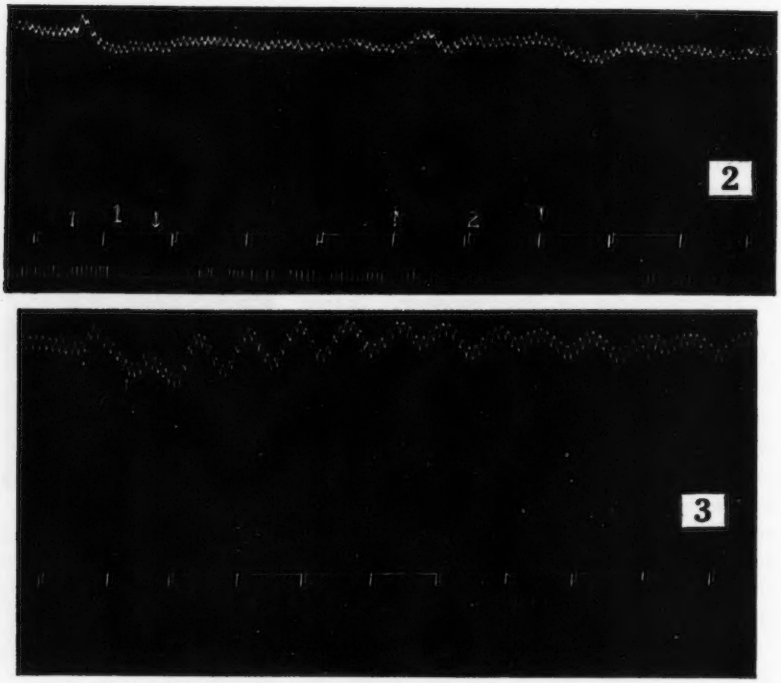


Fig. 2. Female cat, decerebrated and ether discontinued 1½ hours previously. Top record, blood pressure with mercury manometer; middle record, time interval in 30 seconds and zero blood pressure; bottom record, drops of blood leaving the adductor femoris muscle. *1* and *2*, 0.2 cc. adrenalin chloride 1:100,000.

Fig. 3. Male cat decerebrated and ether discontinued 2 hours. Otherwise same as figure 2. *2*. Injection of 0.2 cc. of a 1:100,000 adrenalin chloride.

they usually follow the primary fall and occur during the return in blood pressure to normal. In decerebrated animals in which the anesthesia had been discontinued for some time, the second, third and fourth injections of epinephrin may be followed by no change in blood pressure other than these oscillations. Figure 3 shows such changes. In this animal the ether had been discontinued 2 hours before the first injection was made.

In this animal 0.2 cc. of a 1:100,000 solution of adrenalin chloride caused a fall in blood pressure more marked than that illustrated in figure 1. In addition to the fall, oscillations in blood pressure were noted during the recovery phase. An equal quantity of epinephrin was injected at *2*, illustrated in this figure. The fall in blood pressure is not as great as in the first case but the oscillations in blood pressure are equally marked. Later injections caused no change in blood pressure but the oscillations occurred as before. Here as in figure 2 an increase in blood flow through the skeletal muscle tested is noted. This amounted to 17 drops per 30 seconds.

From the results presented here the vaso-constrictor response to epinephrin appears to be increased following decerebration and withdrawal of the anesthesia. The dilator response however is not lost, as McDonald and Schlapp have suggested.

SUMMARY

1. Epinephrin in small doses causes a fall in blood pressure when injected intravenously in decerebrated cats from which the anesthetic had been withdrawn from 30 minutes to $3\frac{1}{2}$ hours.

2. Decerebration does not alter the vasomotor response to epinephrin in skeletal muscle.

3. Decerebration heightens the vasoconstrictor action of epinephrin.

4. Epinephrin, when injected intravenously in decerebrated animals frequently causes vasomotor variations to an extent not commonly observed in anesthetized cats.

BIBLIOGRAPHY

(1) Oliver and Schäfer: Journ. Physiol., 1895, xviii, 233.
(2) Szymonowicz: Pflüger's Arch., 1896, lxiv, 97.
(3) Cannon and Lyman: This Journal, 1913, xxi, 384.
(4) Dale: Journ. Physiol., 1905, xxxii, 59; Ibid., 1906, xxxiv, 169.
(5) Hartman: This Journal, 1915, xxxviii, 439, 444.
(6) Hartman and McPhedran: This Journal, 1917, xliii, 311.
(7) Hartman and Fraser: This Journal, 1917, xliv, 353.
(8) Hartman: Journ. Pharm. Exper. Therap., 1918, xi, 186.
Hartman and Kilborn: This Journal, 1918, xlv, 111.
Hartman, Kilborn and Fraser: Ibid., 1918, xlvi, 169, 502.
(9) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(10) Gruber: This Journal, 1917, xliii, 530.
(11) Gruber: This Journal, 1918, xlv, 302.
(12) Dale and Richards: Journ. Physiol., 1918–19, lii, 119.
(13) Pearlman and Vincent: Endocrinol., 1919, iii, 121.
(14) McDonald and Schlapp: Journ. Physiol., 1926, lxii, xii.
(15) Gruber: This Journal, 1914, xxxiii, 336; Ibid., 1914, xxxiv, 89.

CHEMICAL CHANGES IN THE BLOOD DURING FASTING AND SUBSEQUENT REFEEDING

II. Inorganic Components

SERGIUS MORGULIS

With the technical assistance of V. L. Bollman

From the Department of Biochemistry, Nebraska College of Medicine

Received for publication November 3, 1927

In a previous paper (1) the changes in composition of the blood of fasting dogs were described from the point of view of its non-protein nitrogenous components. The investigation has been extended further to the inorganic components of the blood. At first whole blood was studied, but it soon became apparent that the experimental results were difficult to interpret owing to the changes in the relative volume of cells and plasma under the conditions of fasting and of subsequent refeeding. In later experiments the analyses were, therefore, carried out on plasma or serum. It was learned from the earlier experience that no noteworthy changes in the inorganic components of the blood appear before inanition has progressed to a degree when the body weight has diminished at least 20 per cent. The analyses were, therefore, limited to blood samples obtained at two stages of inanition: one, about the middle of the fast, when the loss in weight is 20 to 25 per cent; and, again, at a much more advanced stage, when there was a loss of 35 per cent or more. Inasmuch as we were also interested in the changes coincident with refeeding, we avoided unnecessary protraction of the fast to such extreme conditions of debility where the success of refeeding becomes somewhat uncertain. In view of the fact that large amounts of blood were required for the various analyses, the limitation of the number of bleedings in the course of an experiment was very desirable to prevent the occurrence of hemorrhage effects which might tend to obscure the influence of inanition *per se*.

All dogs used in these experiments were carefully selected with regard to size and health, and were kept on the usual laboratory diet for a considerable time before their blood was examined. We employ as our criterion of the availability of an animal for purposes of experimentation the constancy of its body weight over a period of at least one week. The dogs in the mean time become thoroughly inured to the laboratory routine and manipulation, so that they keep perfectly quiet while being bled, without

resort to anesthesia, directly from the heart. The blood was always sampled 24 hours after feeding. Following the first bleeding, to establish the normal composition, the dogs were fed for several days, so as to allow them to recuperate from the effect of the loss of blood, before they commenced their fast. Immediately after the second fasting blood sample was taken the fast was broken and the refeeding commenced.

The blood from the left ventricle of the heart was obtained in two portions: one sample was drawn into a 15 cc. graduated Pyrex centrifuge tube containing a little powdered lithium citrate, the blood being collected under paraffin oil and out of contact with the air, and immediately centrifuged at high speed. The clear plasma was used for determining the total CO_2, calcium and magnesium. The centrifuging was continued until the volume occupied by the cells remained unchanged and the percent of plasma was calculated from the original volume of the blood sample and that represented by the cells. A second, much larger sample of blood was collected in a flask and defibrinated with glass beads. The coagulum was centrifuged off, the clear serum diluted with three volumes of distilled water and deproteinized with one volume of 20 per cent trichloracetic acid. By carrying out the defibrination carefully we have rarely been troubled even with traces of hemolysis. The protein precipitate was centrifuged off and the supernatant fluid was poured through an ashless paper. In this protein-free filtrate we determined sodium, potassium, chlorides, inorganic phosphate and frequently also calcium and magnesium.

We have made many parallel determinations of calcium and magnesium on both serum and plasma, and the differences between the two determinations when such did occur were entirely within the experimental error of the analyses. Such comparative studies were made not only on serum from defibrinated blood but also from blood which was allowed to clot spontaneoulsy, and on normal and fasting bloods. We feel, therefore, justified in referring in this paper indifferently to serum or plasma calcium. Very frequently we had analyses on both, but whenever not enough material was available for all determinations the calcium and magnesium were determined only in the plasma.

METHODS OF ANALYSIS. The analyses were made by procedures generally accepted in micro-chemical work, with such slight modifications as our experience has proved desirable to obtain accurate results.

Sodium. The sodium was determined in the trichloracetic filtrate from blood serum by the titrimetric method of Kramer and Gittelman (2). Special attention must be paid to the complete removal of the trichloracetic acid. An aliquot of the filtrate was evaporated with 5 cc. concentrated nitric acid, the residue was taken up in a few drops of water and again evaporated, repeating this several times. The final solution of the residue should turn alkaline on the addition of a drop of 10 per cent K O H. Apart

from running the usual blank determination on the reagents, the accuracy of the analyses was frequently checked by determinations on solutions of known NaCl content.

Potassium. The potassium was determined by the method of Kramer and Tisdall (3). To prevent losses of the fine precipitate in the process of washing we used water containing 0.5 cc. caprylic alcohol per liter. The factor 0.071 for converting the $KMnO_4$ titration figure into milligrams of potassium is an empirical value and, at least in our hands, was found to vary with each set of reagents. Inasmuch as 5 per cent variation may result from this source we made it a practice to evaluate this factor every time new reagents were prepared from quadruplicate analyses with known amounts of potassium chloride.

Calcium. The calcium in plasma was determined by the procedure recommended by Clark and Collip (4). When the determination was also carried out in the filtrate from deproteinized serum, the reaction was adjusted using methyl red previous to the precipitation of the calcium.

Magnesium. The magnesium was determined in plasma from which the calcium was precipitated or in the trichloracetic acid filtrate from serum after a similar complete removal of calcium. The determination was made by Brigg's procedure (5). This colorimetric method, however, leaves much to be desired from the standpoint of accuracy. The magnesium results are affected by two conditions. First, by lack of proportionality between the color developed and the amount of magnesium in the solution. This is easily overcome since readings which do not vary by more than 10 per cent from the standard (i.e., 18 to 22 mm. if the standard is set at 20 mm.) give good checking results, and the amount analyzed is adjusted correspondingly. The second condition is more serious and may be the cause of more serious error. To bring down the precipitate of the NH_4MgPO_4 it is necessary to rub the sides of the tube with a glass rod. Leaving out this part of the procedure may give rather low results, while the rubbing is itself responsible for a certain amount of color development. This extra color may possibly be due to some silicate and we found that silicate does produce the same color with the molybdate reagent as does phosphate. We have made various attempts to standardize the analysis with this in view but finally decided on the advisability of retaining the practice of rubbing down the sides and introducing an empirical correction of 10 per cent.

Phosphate. The inorganic phosphate (6) was determined by the Brigg's modification of the Bell-Doisy method in the protein-free filtrate from serum. We found it desirable, also in this determination, to adjust the amount of filtrate analyzed so that the color of the standard was matched within a narrow range.

Chlorides. The chlorides were determined in the trichloracetic filtrate

by the titration recommended by Whitehorn (7). The method was checked by analyses with known amounts of sodium chloride in the presence of trichloracetic acid and also by recovering sodium chloride added to the serum filtrates. The trichloracetic acid must be fresh.

TABLE 1

Composition of whole blood from normal dogs

DOG NUMBER	MGM. PER 100 CC. BLOOD						PER CENT OF PLASMA
	Na	K	Ca	Mg	Cl	P	
1 a	299	29.7	5.9	5.0	296	3.6	51.7
2 a	238	31.8	7.0	4.1	289	4.5	54.2
3 a	275	35.8	4.9	4.5	299	4.2	56.6
4 a	267	27.7	4.4	4.5	299	4.9	53.5
5 a	252	27.1	4.6	4.7	280	2.5	53.3
6 a	279	32.8	6.5	3.0	221	4.6	
7 a	255	35.2	6.5	2.4	293	4.1	
8 a	300	35.5	6.8	3.4	296	3.9	
Averages....	271	32.0	5.8	4.1	284	4.0	53.6

TABLE 2

Composition of serum from normal dogs

DOG NUMBER	MGM. PER 100 CC. SERUM						PLASMA CO_2 VOLUME	PER CENT OF PLASMA
	Na	K	Ca	Mg	Cl	P		
							per cent	
1	366	23.2	11.3	2.5	410	4.8	51.6	50.0
2	367	24.4	11.4	2.4	403	4.9	54.0	50.2
3	361	25.0	11.5	2.4	410	5.0	48.8	53.6
4	372	21.7	10.9	2.2	432	4.5	47.0	55.1
5	420	19.3	10.6	2.8	448	4.1	57.2	
6	363	18.7	10.9	2.7	461	2.9	45.1	
7	349	23.3	11.7	1.8	426	4.8	46.2	55.2
8	342	21.7	10.7	2.0	411	3.7	46.0	50.0
9	346	20.8	11.0	2.0	420	4.0	48.3	52.3
10	346	22.7	11.7	1.8	427	4.3	45.2	52.6
11	353	17.8	10.4	2.4	432	3.4	51.6	
12	348	22.8	10.6	2.2	417	5.1	47.9	55.6
13	349	18.3	11.0	2.8	420	3.7	49.8	51.0
14	343	20.8	10.8	2.2	439	4.9	51.5	54.6
Averages..	359	21.5	11.0	2.3	423	4.3	49.3	52.7

Total carbon dioxide. The CO_2 combining power of true plasma was determined with the Van Slyke and Neill constant volume apparatus (8).

EXPERIMENTAL RESULTS. Although the changes in the inorganic components of serum and plasma will be reported in this paper, the results of

analyses on whole blood of normal dogs are presented in table 1. With the aid of the data on the plasma volume and the composition of the plasma under normal conditions, given in table 2, the electrolyte content of the red cells has been calculated. These data give further evidence of what has already been demonstrated for human blood (9), namely, that the red cells of dog blood are likewise practically free from calcium (except for such minute traces as may be assumed to adhere to their surface) which is evidently a component of plasma only. We found that the calcium of whole blood varies from 4.4 to 7.0 mgm. per 100 cc. with an average value of 5.8 mgm., which is in excellent agreement with the analytical results reported by Cameron and Moorehouse (10). On the contrary, the magnesium is confined chiefly to the cells where the concentration is about 2.7 times as great as in plasma. The concentrations of sodium and of potassium in serum and cells, respectively, bear a reciprocal relation to each other, the ratio of serum sodium to cell sodium being 2.1:1, while that for potassium is 1:2.1. For purposes of comparison we give below the composition of cells and serum of the dog (our analyses) and those for the cat, based on the experimental data of Baumann and Kurland (11).

MGM. PER 100 CC.		Na	K	Ca	Mg	Cl	P
Dog	Serum	354	21.5	11.0	2.3	423	4.3
	Cells	178	44.7	(0.2)	6.1	119	3.7
Cat	Serum	375	19.0	10.2	2.4	415	5.8
	Cells	325	33.3	3.4	5.0	279	8.1

In the tables 3, 4 and 5 are recorded the results of the analyses of blood serum from dogs which lost 20 to 25 per cent, and 35 per cent or more of their initial weight as a result of inanition, and finally after one week of refeeding.

The sodium and potassium content of the serum shows practically no change in the early stages of inanition (up to a loss in weight of 20 per cent) but diminishes in the more advanced stage. If the sodium and potassium level is low at the commencement of the fast, it rises at first and then falls as the 35 per cent loss in weight is approached. On refeeding the sodium level is rapidly reëstablished, but if towards the end of the fast it increased above the initial value, as occasionally happens, it diminishes to the pre-fasting value on refeeding. The potassium continues to fall during the first week of refeeding.

The calcium content of the serum does not change appreciably until the fasting has progressed beyond the 20 per cent loss in weight, and the pre-fasting level is rapidly reached upon refeeding. The magnesium shows a tendency to rise during the early part of the fast, followed by a drop in

the more advanced condition without, however, falling below the original value. There is a rapid return to the pre-fasting level on refeeding.

TABLE 3

Composition of serum from fasting dogs

DOG NUMBER	LOSS IN WEIGHT	MGM. PER 100 CC. SERUM						PLASMA CO_2 VOLUME	PER CENT OF PLASMA
		Na	K	Ca	Mg	Cl	P		
	per cent							*per cent*	
1	19.3	334	22.7	10.2	2.7	409	4.0	51.9	41.7
2	25.5	348	21.6	11.8	2.6	423	4.0	48.7	42.1
4	21.5	344	21.7	11.0	2.0	428	5.0	46.8	49.5
5	21.0	398	22.5	10.0	2.0	435	3.6	54.1	43.1
7	21.5	373	25.1	10.5	2.2	420	4.3	40.1	49.6
9	23.1	372	24.1	10.6	2.4	425	3.9	38.3	50.4
10	25.5	375	21.1	10.0	2.4	426	3.2	45.2	44.9
12	23.2	376	23.5	9.7	2.5	457	4.4	48.2	45.5
13	22.0	330	19.4	10.6	2.8	421	2.8	52.0	48.9
14	20.9	349	21.6	11.3	2.5	417	3.4	56.4	48.1
Averages.....		360	22.3	10.6	2.4	426	3.9	48.2	46.4

TABLE 4

Composition of serum from fasting dogs

DOG NUMBER	LOSS IN WEIGHT	MGM. PER 100 CC. SERUM						PLASMA CO_2 VOLUME	PER CENT OF PLASMA
		Na	K	Ca	Mg	Cl	P		
	per cent							*per cent*	
1	35.9	374	18.2	10.5	1.8	392	3.0	57.3	44.1
2	40.4	389	18.1	9.2	2.4	390	3.8	56.5	48.4
3	36.0	367	20.6	9.9	1.8	412	4.2	47.7	41.0
4	40.3	364	16.4	9.6	2.2	404	5.1	39.5	51.3
5	37.6	410	20.1	9.6	2.1	425	3.7	53.4	45.3
7	39.5	336	21.3	9.8	1.8	427	5.6	40.8	
8	38.0	338	20.8	9.9	2.1	425	3.4	48.2	47.2
9	40.7	345	21.8	8.6	2.3	425	3.5	47.8	49.4
10	43.5	332	20.0	10.2	2.2	411	4.1	50.2	
11	35.8	324	20.0	8.0	2.3	389	4.6	49.4	48.4
13	35.0	319	23.2	10.6	2.4	370	2.8	58.9	40.3
14	31.2*	323	22.1	10.0	2.5	392	3.0	56.9	50.0
Averages.....		354	20.0	9.6	2.2	405	3.9	50.6	46.5

* Not included in the average.

The chlorides diminish definitely only during the advanced stage of fasting. In the early part the chlorides may either increase or decrease, if the pre-fasting chloride level is either lower or higher than the average

value. The normal level is quickly reëstablished on feeding. Inorganic phosphates diminish more rapidly during the first part of the fast than during the later stages, and on refeeding increase rapidly. Occasionally, however, when the phosphates during fasting have been increasing, there is a sharp drop on refeeding. The CO_2 content is practically unaltered during the early stage of fasting while in the more advanced stage, as a rule, it increases. On refeeding, the CO_2 soon returns to the normal level.

A word with respect to the plasma volume will not be amiss. The references in literature to normal dog plasma show an extreme degree of variability. In our experience, judging the normal condition by the criterion of weight constancy, the plasma volume for different dogs varies within the limits of 50 to 56.6 per cent, with an average of about 53 per cent.

TABLE 5

Composition of serum from refed fasting dogs

DOG NUMBER	GAIN IN WEIGHT	MGM. PER 100 CC. SERUM						PLASMA CO_2 VOLUME	PER CENT OF PLASMA
		Na	K	Ca	Mg	Cl	P		
	per cent							*per cent*	
1	34.3	419	19.7	11.6	2.3	420	4.6	55.8	54.6
2	30.5	375	19.3	11.2	1.8	396	3.0	56.0	66.0
3	33.1	418	19.1	11.7	2.4	420	5.2	45.8	59.5
5	18.7	384	18.6	10.4	1.8	427	3.6	48.3	60.2
7	20.8	343	19.7	10.3	2.0	436	5.5	45.8	78.2
8	52.3	345	22.0	10.2	2.7	424	5.1	51.9	67.2
10	35.8	357	28.1	10.3	2.5	422	6.0	51.4	62.7
11	19.3	338	22.2	10.3	2.0	406	5.7	54.1	65.3
13	16.0	339	19.7	9.8	2.4	439	2.7	39.8*	50.7
14	11.4	334	17.5	10.2	2.5	431	3.4	51.8	61.1
Averages.....		365	20.6	10.6	2.2	422	4.5	51.2	62.5

* Dog struggled during bleeding.

During fasting, with or without water, the average plasma volume is 46 per cent (variations 41.7 to 51.3 per cent). But whenever a starved dog is fed again there is a rapid and very remarkable increase in the plasma volume so that the extreme fluidity of the blood of refed dogs at once attracts attention. Under this condition the plasma volume may even become 80 per cent. The reports in the literature give a range of plasma volume for normal dogs from 42 to 74 per cent. It is very doubtful if the values really represent the normal condition in the sense of a nutritional balance, as in the case of our dogs, and it is not improbable that the very high results were found on dogs which reached the laboratory in a starved condition and were experimented on soon after being refed.

DISCUSSION. Surveying the results of this study of the inorganic com-

ponents of serum from dogs undergoing a long fast it is obvious that the electrolyte composition shows remarkable stability and does not betray any clear-cut changes until a very advanced stage of inanition is reached and the body weight diminishes by 35 per cent or more. However, the variations observed in individual animals are such that one is justified in speaking only of tendencies rather than definite changes, and no statement can be made which will invariably apply to all animals at a given stage of inanition. Owing to the fact that at least up to the time of about 22 per cent loss in weight the range of variation remains the same as it was in normal dogs, the average values for the various electrolytes likewise remain practically normal. Nevertheless, when one examines the serum from a number of animals, certain trends of alteration can be recognized unmistakably, as, for instance, the definite diminution in calcium and chloride concentration. A few days of refeeding, however, suffice to bring these back to practically a pre-fasting level. This constancy of the composition of the blood serum is revealed even more strikingly from a study of the ratios between different electrolytes. Thus, the K/Ca ratio for normal dog serum is 1.95 (1.7–2.2) but increases to 2.11 (1.8–2.4) during the first half of fasting owing to the more rapid decrease in the calcium than in the potassium. The ratio remains almost the same in the more advanced stage of fasting (2.08) because both calcium and potassium diminish at about the same ratio during the later stage of inanition. On refeeding the original pre-fasting ratio is at once reëstablished. It is also interesting to compare the Ca $\times$ P product at different phases of fasting since a favorable effect on the healing of rickets has been attributed to inanition. Howland and Kramer (12) have shown that values less than 40 usually coincide with rachitic changes in the bones. In our dogs the value of this product for normal serum was on the average 47.4, and even at the time of a 22 per cent loss in weight was still 41, i.e., outside the rachitic zone. In the advanced stages, however, it drops to 37.5 which is already within the rachitic zone of Howland and Kramer. After one week of refeeding the value of the Ca $\times$ P product returns to its normal value again. We find, therefore, no indication from this relationship as to the cause of the curative action of fasting on rickets. Cavins (13) reports that the inorganic phosphate in serum of rachitic rats submitted to fasting rises very markedly and to unusually high values, to which fact he apparently attributes the healing of the disease. In rachitic rats the blood phosphate diminishes greatly. In normal fasting rats the phosphate of the blood serum also increases but not anywhere to the same extent as in the rachitic animals. In our experiments on fasting dogs we have never seen any striking changes in the serum phosphates, the tendency being towards a falling off in concentration as the fast progresses. The only exception to this is found in the event of some intervening infection, in

which cases very marked rises (three- to fourfold) in the serum phosphate have been noted together with similar striking increases in potassium and magnesium concentration. In some instances these changes are merely a part of the general increase in all blood electrolytes, and are obviously associated with a serious kidney involvement, but we have also seen cases where the increase was limited to phosphates, potassium and magnesium alone. In dogs, going through a prolonged fast uncomplicated by disease, we have not found evidence of a rise in phosphates. It is, of course, possible that the results observed by Cavins in the fasting rachitic rats, whose resistance is naturally greatly reduced, may likewise have been due to the effect of some infection rather than to the inanition.

The fall in calcium is very noteworthy as it is probably one of the most definite changes occurring during inanition. Goto's results (14) on the blood of starving rabbits are not as clear as our own obtained on dogs because he did not follow the changes in the same animal at different stages of inanition but studied its effect in rabbits sacrificed at different times in the course of fasting. His analytical results betray a range of variation (the calcium of the blood of normal rabbits is given as 4.7 to 11.3 mgm. per 100 cc.!) which casts a certain amount of doubt upon their accuracy. Besides, in view of such a wide diversity of the normal values, the variations in the calcium content of the blood from fasting rabbits lose much of their significance. However, even Goto's findings indicate a general tendency of the blood calcium to diminish during fasting. It is well to inquire as to the portion of the serum calcium which undergoes diminution during inanition. As is well known, only a part of the blood calcium is in diffusible form (according to Cameron and Moorehouse (10) 53 per cent of the plasma calcium in normal dogs) while the rest is in combination with protein and in some other non-diffusible form. In our earlier experiments when the analyses were made on whole blood, the calcium in the advanced stages of fasting was found to fall to extremely low values (3 to 4 mgm. per 100 cc. blood) such as are usually associated with manifestations of severe tetany. Nevertheless, we have never seen convulsive attacks in our starved dogs. Calcium determinations which have been made simultaneously on serum from clotted blood or on citrated plasma gave concordant results, and while they showed that the calcium was below normal the diminution was not as great as in the case of the whole blood of the same animal. Indeed, we found that the reduction of the calcium in the whole blood is an exaggeration of the plasma calcium reduction due to the change in plasma volume percent during fasting. This is well illustrated by the following example where the calcium content of whole blood as determined by direct analysis and as calculated from the calcium content of the plasma and the relative plasma volume of the blood are given.

CONDITION OF DOG	PLASMA VOLUME	Ca PER 100 CC. IN MGM.			Ca PER 100 CC. WHOLE BLOOD (CALCULATED)
		Serum	Plasma	Whole blood	
	per cent				
Normal	51.7	11.0	11.1	5.9	5.8
Loss 13 per cent	45.6	10.0	10.1	4.1	4.6
Loss 34 per cent	35.1	10.1	10.2	3.3	3.5
Refed	51.5		10.2	5.0	5.2
Refed	54.6		11.1	6.0	6.3

As can be seen from these data, on the assumption that all the calcium is present in the plasma, as was found to be true for normal dog blood, the calculated amount of calcium in whole blood and that found by actual analysis are in excellent agreement. It also shows how it came about that the starved dogs have not developed tetany in spite of the fact that the whole blood calcium dropped as low as it does in cases of extreme tetany. The plasma calcium had actually diminished only about 10 per cent in this animal. In as much as in some of our starved dogs the calcium content of the plasma diminished even 20 per cent without causing at the same time convulsive attacks, we are justified in assuming that the reduction in calcium during fasting and during tetany must have a different origin. If we accept the view of Salversen and Linden (15) we should be led to conclude that only the non-diffusible and un-ionized moiety of the plasma calcium must be diminished. The fact that the protein content of serum in fasting dogs may be decreased, as shown by Polányi (16) would then seem to offer an explanation for the calcium loss. It is also well to note that Csapó and Faubl (17) found that calcium combined more readily with the finely dispersed serum protein (albumin) than with the more coarsely dispersed ones (globulin, fibrinogen) the ratio of calcium in combination with 100 grams of each of these proteins being 78 : 38 : 11.5. Robertson reports an increase in the globulin/albumin ratio in the serum of starved dogs. Even disregarding the still mooted question of the change in the protein content of fasting serum, the greater proportion of globulin with a low combining power for calcium may easily account for a loss of 1 to 2 mgm. of calcium per 100 cc. serum, which is about the magnitude of the decrease found in our experiments.

The change observed in fasting dogs and in dogs suffering from parathyroid tetany is obviously of a different nature, and this is brought out clearly by a comparison of our results with those of Cameron and Moorehouse. Taking their data for calcium in the serum of their dogs 10, 11 and 12 with marked attacks of tetany we may calculate the calcium content of whole blood, using the corresponding data for plasma volume per cent as was also done in the table above. Unfortunately their data for

the plasma volume per cent show much variation, but using the average from the several determinations we get the following results.

DOG	CONDITION	MGM. Ca PER 100 CC. WHOLE BLOOD	
		Found	Calculated
10	Normal	6.6	5.1
	Tetany	4.4	2.9
	Tetany	4.0	2.6
11	Normal	6.7	5.5
	Tetany, moderate	6.1	3.1
	Tetany, acute	?	3.1
	Tetany, extreme	5.0	2.1
12	Normal	7.0	6.8
	Tetany, moderate	4.6	3.0
	Tetany, acute	5.3	2.4

The lack of agreement between the analytical and calculated data for whole blood calcium in dogs suffering from tetany is thus in marked contrast with the previous record showing the very good agreement in these values for starved dogs. Obviously the assumption that the calcium is present only in the plasma but not in the blood cells does not hold for animals in tetany, and the calculated blood calcium is, therefore, much lower than the calcium directly determined by analysis. It would seem, therefore, that some of the calcium must be transferred to the cells. Cameron and Moorehouse argue from their very interesting experiments that tetany is associated with a partial or even complete loss of a non-diffusible organic compound of calcium since in extreme tetany the calcium content of serum, blood and cerebrospinal fluid (the latter presumably representing a dialyzate of the blood) becomes the same, and must therefore represent calcium only in a diffusible state. On purely theoretical grounds it is difficult to conceive how the concentration of calcium in whole blood and serum may become equal unless the cell does become completely permeable to the calcium and the latter is evenly distributed in both the cellular and in the fluid phase of the blood. At any rate, the development of tetany in parathyroidectomized dogs and its failure to develop in starved dogs even in moderate form suggests that probably some physiological disturbance apart from the change in calcium is essential in the development of the disease.

The more or less decided diminution in chlorides observed in very advanced stages of fasting is also worth considering. Goto (18) claims that in fasting rabbits the blood chlorides increase reaching a maximum concentration ten days after the commencement of inanition. We have rarely

seen a rise in serum chlorides in our fasting dogs. During the first half of the fast, i.e., up to the time of about 20 per cent loss in weight, the serum chlorides may be said to be unchanged in view of the wide variation prevalent among different animals. When, however, the body weight has already diminished by 35 per cent or more the serum chloride practically without exception diminishes, though the extent of this reduction varies greatly for different dogs. Since the excretion of chlorides in the course of inanition decreases almost to the vanishing point in the later stages, this drop in serum chlorides cannot be attributed to an increased elimination and some other mechanism must be operative in producing this marked decrease. A suggestion of the possible cause for this is obtained from the fact that the bicarbonate increases at the same time as the chlorides diminish. In fact, in the few instances where the serum chlorides at the conclusion of the experiment did not diminish or have actually increased the bicarbonate likewise underwent little alteration, and, furthermore, where the chlorides dropped very markedly the bicarbonate rise was very definite. We may perhaps be dealing here with a condition not unlike that described by Haden and Orr (19) in the case of intestinal obstruction where some toxic substance, possibly a proteose, apparently combines with chloride as a means of detoxication and sets free sodium, thus leading to an increase in the bicarbonate content of the blood. In the advanced stages of fasting, as in the case of intestinal obstruction, there is an accumulation of non-protein nitrogenous products in the blood, as was shown in previous papers (1, 20). It seems likely, therefore, that with the progress of fasting owing either to the increasing permeability of the digestive tract or to some abnormal cleavage processes in the protein catabolism toxic substances are formed which affect the chemical reactions in the blood much in the same way as does intestinal intoxication.

In studies on human subjects it was found that the alkalinity of the blood diminishes during fasting, though this changes little after an initial drop observed after the first few days of inanition. Asada (21) experimenting on rabbits with the Van Slyke method shows that the alkaline reserve of the blood diminishes regularly in the course of inanition and finally falls to about one-half its normal value in the advanced stages. The results of our study of the carbon dioxide content of the plasma fail to corroborate these findings, though occasionally the alkaline reserve of our fasting dogs did diminish. Usually, however, we found that there is no appreciable alteration even up to the time when the organism had lost 20 per cent in weight, and in the very advanced stage of inanition there is a very pronounced tendency for the alkaline reserve to increase.

the plasma volume per cent show much variation, but using the average from the several determinations we get the following results.

DOG	CONDITION	MGM. Ca PER 100 CC. WHOLE BLOOD	
		Found	Calculated
10	Normal	6.6	5.1
	Tetany	4.4	2.9
	Tetany	4.0	2.6
11	Normal	6.7	5.5
	Tetany, moderate	6.1	3.1
	Tetany, acute	?	3.1
	Tetany, extreme	5.0	2.1
12	Normal	7.0	6.8
	Tetany, moderate	4.6	3.0
	Tetany, acute	5.3	2.4

The lack of agreement between the analytical and calculated data for whole blood calcium in dogs suffering from tetany is thus in marked contrast with the previous record showing the very good agreement in these values for starved dogs. Obviously the assumption that the calcium is present only in the plasma but not in the blood cells does not hold for animals in tetany, and the calculated blood calcium is, therefore, much lower than the calcium directly determined by analysis. It would seem, therefore, that some of the calcium must be transferred to the cells. Cameron and Moorehouse argue from their very interesting experiments that tetany is associated with a partial or even complete loss of a non-diffusible organic compound of calcium since in extreme tetany the calcium content of serum, blood and cerebrospinal fluid (the latter presumably representing a dialyzate of the blood) becomes the same, and must therefore represent calcium only in a diffusible state. On purely theoretical grounds it is difficult to conceive how the concentration of calcium in whole blood and serum may become equal unless the cell does become completely permeable to the calcium and the latter is evenly distributed in both the cellular and in the fluid phase of the blood. At any rate, the development of tetany in parathyroidectomized dogs and its failure to develop in starved dogs even in moderate form suggests that probably some physiological disturbance apart from the change in calcium is essential in the development of the disease.

The more or less decided diminution in chlorides observed in very advanced stages of fasting is also worth considering. Goto (18) claims that in fasting rabbits the blood chlorides increase reaching a maximum concentration ten days after the commencement of inanition. We have rarely

seen a rise in serum chlorides in our fasting dogs. During the first half of the fast, i.e., up to the time of about 20 per cent loss in weight, the serum chlorides may be said to be unchanged in view of the wide variation prevalent among different animals. When, however, the body weight has already diminished by 35 per cent or more the serum chloride practically without exception diminishes, though the extent of this reduction varies greatly for different dogs. Since the excretion of chlorides in the course of inanition decreases almost to the vanishing point in the later stages, this drop in serum chlorides cannot be attributed to an increased elimination and some other mechanism must be operative in producing this marked decrease. A suggestion of the possible cause for this is obtained from the fact that the bicarbonate increases at the same time as the chlorides diminish. In fact, in the few instances where the serum chlorides at the conclusion of the experiment did not diminish or have actually increased the bicarbonate likewise underwent little alteration, and, furthermore, where the chlorides dropped very markedly the bicarbonate rise was very definite. We may perhaps be dealing here with a condition not unlike that described by Haden and Orr (19) in the case of intestinal obstruction where some toxic substance, possibly a proteose, apparently combines with chloride as a means of detoxication and sets free sodium, thus leading to an increase in the bicarbonate content of the blood. In the advanced stages of fasting, as in the case of intestinal obstruction, there is an accumulation of non-protein nitrogenous products in the blood, as was shown in previous papers (1, 20). It seems likely, therefore, that with the progress of fasting owing either to the increasing permeability of the digestive tract or to some abnormal cleavage processes in the protein catabolism toxic substances are formed which affect the chemical reactions in the blood much in the same way as does intestinal intoxication.

In studies on human subjects it was found that the alkalinity of the blood diminishes during fasting, though this changes little after an initial drop observed after the first few days of inanition. Asada (21) experimenting on rabbits with the Van Slyke method shows that the alkaline reserve of the blood diminishes regularly in the course of inanition and finally falls to about one-half its normal value in the advanced stages. The results of our study of the carbon dioxide content of the plasma fail to corroborate these findings, though occasionally the alkaline reserve of our fasting dogs did diminish. Usually, however, we found that there is no appreciable alteration even up to the time when the organism had lost 20 per cent in weight, and in the very advanced stage of inanition there is a very pronounced tendency for the alkaline reserve to increase.

CONCLUSION

As a general conclusion from our experiments we may state that the electrolyte composition of the dog's blood is maintained with remarkable tenacity showing little alteration in the course of inanition. The striking changes usually appear in the more advanced condition of starvation. The diminution in the chlorides is shown to be associated with a more or less pronounced rise in the bicarbonate content of the plasma, and this may probably be due to a toxicosis developing at that stage of fasting. The effect is not caused by any degenerative process in the organism since it disappears after a few days of renewed feeding. Calcium likewise undergoes considerable diminution in the course of inanition, and as is shown by comparative studies of the calcium of whole blood, plasma and serum, at all stages of fasting it is invariably found only in the fluid portion of the blood. The lowering of the calcium content may perhaps be due to a shifting in the globulin/albumin ratio in the serum during fasting.

BIBLIOGRAPHY

(1) Morgulis, S. and A. C. Edwards. This Journal, 1924, lxviii, 477.
(2) Kramer, B. and J. Gittelman. Journ. Biol. Chem., 1924, lxii, 353.
(3) Kramer, B. and F. F. Tisdall. Journ. Biol. Chem., 1921, xlvi, 339.
(4) Clark, E. P. and J. B. Collip. Journ. Biol. Chem., 1925, lxiii, 461.
(5) Briggs, A. P. Journ. Biol. Chem., 1922, lii, 349.
(6) Briggs, A. P. Journ. Biol. Chem., 1924, lix, 255.
(7) Whitehorn, J. C. Journ. Biol. Chem., 1921, xlv, 449.
(8) Van Slyke, D. D. and J. M. Neill. Journ. Biol. Chem., 1924, lxi, 523.
(9) Kramer, B. and F. F. Tisdall. Journ. Biol. Chem., 1922, liii, 241.
(10) Cameron, A. T. and V. H. K. Moorehouse. Journ. Biol. Chem., 1925, lxiii, 687.
(11) Baumann, E. J. and S. Kurland. Journ. Biol. Chem., 1927, lxxi, 281.
(12) Howland, J. and B. Kramer. Monatschr. Kinderheilk., 1923, xxv, 279.
(13) Cavins, A. W. Journ. Biol. Chem., 1924, lix, 237.
(14) Goto, K. Biochem. Journ., 1922, i, 321.
(15) Salversen, H. A. and G. C. Linder. Journ. Biol. Chem., 1924, lviii, 617, 635.
(16) Polányi, M. Biochem. Zeitschr., 1911, xxxiv, 192.
(17) Csapó, J. und J. Faubl. Biochem. Zeitschr., 1924, cl, 509.
(18) Goto, K. Tohoku Journ. Exper. Med., 1922, iii, 195.
(19) Haden, R. L. and T. G. Orr. Journ. Exper. Med., 1923, xxxvii, 365; xxxviii, 55.
(20) Morgulis, S. Fasting and undernutrition. E. P. Dutton & Co., 1923.
(21) Asada, H. This Journal, 1919, l, 1.

THE PHOTODYNAMIC ACTION OF HEMATOPORPHYRIN

ETHELBERTA NORRIS RASK AND W. H. HOWELL

From the Deprtment of Physiology, School of Hygiene and Public Health, Johns Hopkins University

Received for publication December 9, 1927

The photodynamic action of various fluorescent dyes has attracted attention since Raab (1900) observed that acridin is fatal to paramecia in the light but not in the dark. Hausmann (1908, 1911, 1914) was the first to show that hematoporphyrin exhibits this action and that in the presence of light it not only causes the destruction of paramecia and red corpuscles, but will bring about the death of a mammal (mice). He found that 2 mgm. injected subcutaneously might be fatal to a mouse if it was exposed to light, while a much larger dose was harmless if the animal was kept in the dark. While he described in detail the symptoms exhibited by the sensitized animal, he was not able to find the cause of its lethal action. Pfeiffer (1911) has demonstrated a similar effect upon guinea pigs and Meyer-Betz (1913) in experiments made upon himself showed that the symptoms in man are the same as those exhibited by the lower animals. The train of symptoms depends upon the dose and upon the intensity of the light. Usually the injection is followed quickly by an erythema and edema of the parts of the skin exposed to the light, and the animal shows great restlessness, hyperpnea and signs of strong cutaneous irritation. The symptoms increase in severity, although the edema may disappear and the erythema be replaced by a cyanosis, but if the dose is fatal the animal eventually falls into a coma and dies. If the dose is not fatal the animal will exhibit necrotic areas on the parts of the skin that have been most exposed to the light. Autopsies show no obvious lesions that might account for the sudden death of the animal. The known hemolyzing action of hematoporphyrin and light upon washed red corpuscles might suggest that a similar effect takes place in the blood of an exposed animal, but all observers agree that this is not the case. Under normal conditions the red corpuscles of the circulating blood are protected, as will be explained below. The experiments described in this paper were undertaken mainly with the intention of studying the phenomenon of sensitization on larger animals, hoping thereby to throw some light on the cause of the fatal effect.

PREPARATION OF THE HEMATOPORPHYRIN. Since difficulty was experienced in the beginning in obtaining good preparations of hematoporphyrin, the method finally found to be successful is given in some detail. Crystal-

line hematoporphyrin hydrochloride was used prepared in general according to the directions given by Nencki and Zaleski (1900). The procedure followed consisted of the following steps:

1. Preparation of hemin from whole blood or dried hemoglobin, preferably the latter.

2. Separation of iron from the hemin and conversion of the iron-free hemin into crystalline hematoporphyrin hydrochloride.

1. *Preparation of hemin.* Mörner's (1896) method, with slight modifications, was employed in the preparation of hemin. When whole blood was used as a starting material, the procedure was as follows: Three liters of water were added to one liter of defibrinated and strained blood. This solution was heated to boiling and dilute sulphuric acid was added slowly until the precipitate filled the liquid and formed a jelly-like mass. It was then filtered through five or six layers of washed cheese cloth, washed with water and squeezed strongly. Since a large quantity of fresh blood is difficult to work with, dried hemoglobin was substituted as the starting material. This was found more satisfactory than the fresh blood. One pound of hemoglobin (Armour's) was dissolved in ten liters of water. Convenient portions (2½ liters) of this were heated to boiling with the addition of a few drops of 1 per cent sulphuric acid. The precipitate which formed was filtered through cheese-cloth, washed with water and squeezed as strongly as possible. It was then divided into four equal portions. Each portion was treated in the same way as the precipitate obtained from the one liter of fresh blood; viz., the precipitate was rubbed up with 500 cc. of 95 per cent alcohol, strained and squeezed strongly. A mixture was then made of 20 cc. of concentrated sulphuric acid and 20 cc. of 95 per cent alcohol and added to 1750 cc. of 95 per cent alcohol. The residue was thoroughly rubbed up in this mixture and allowed to stand for one hour. It was then filtered through filter paper. For convenience the filtrate was divided into two equal parts and each part was heated to boiling on an electric heater. When boiling, a mixture of 10 cc. of concentrated hydrochloric acid and 10 cc. of 95 per cent alcohol was added. The flask was well shaken and cooled in water. It was allowed to stand two days, after which most of the supernatant liquid was carefully poured off. The remaining liquid which contained the crystals of hemin was centrifugalized. The crystals obtained were washed with water and dried in vacuo.

2. *Preparation of hematoporphyrin.* Hematoporphyrin was prepared by the following procedure, which is a slight modification of that suggested by Nencki and Zaleski. Five grams of powdered hemin were added, small portions at a time, to 75 cc. of hydrobromic acetic acid.[1] The bottle was

[1] The hydrobromic acetic acid solution was prepared by passing gaseous hydrogen bromide into glacial acetic acid at room temperature for a few minutes. The container was then immersed in ice water and the passing of the hydrogen bromide was

gently shaken during the process. It was then stoppered and allowed to stand for three days at room temperature. During this time the bottle was occasionally shaken. When the hemin was completely dissolved it was poured into 600 cc. of water, and allowed to stand from three to six hours. It was then filtered and 10 per cent sodium hydroxide added to the filtrate until an abundant precipitate had formed and the solution was nearly colorless. It was then filtered. The precipitate was washed with water until the filtrate gave no reaction with silver nitrate. The moist precipitate was then digested on a water bath with 2 per cent sodium hydroxide until it was dissolved. The solution was diluted with an equal volume of water and the oxide of iron filtered off. Dilute acetic acid (acetic acid 1 vol.; water 2 vol.) was added to the filtrate until it was distinctly acid to litmus and the solution was nearly colorless. The precipitate formed was separated by centrifugalization and then washed with water until it showed signs of going into a colloidal solution. The moist precipitate was dissolved in 1 per cent hydrochloric acid on a water bath, care being taken that the temperature was not above 70 degrees. The solution was filtered warm, and then evaporated down to a small volume in vacuo over concentrated sulphuric acid.

The crystalline sediment was washed quickly with a small amount of 10 per cent hydrochloric acid and then dissolved in warm 1 per cent hydrochloric acid. After filtering the solution, concentrated hydrochloric acid was added in an amount equal to one-tenth of the volume. The solution was then again crystallized by evaporation in vacuo. Some days are required to evaporate the liquid in vacuo over sulphuric acid. This process can be hastened by bubbling air which has been purified and dried by passing through sulphuric acid, through the solution. Sometimes the liquid was evaporated under an electric fan under a hood. The obvious objection to this method is the possibility of getting dust in the solution. However, experience has shown that this method is quite satisfactory when it is desirable to shorten the process as much as possible. After the second crystallization the material is kept in a desiccator in the dark until used.

EXPERIMENTAL WORK. The effects of the hematoporphyrin were observed upon the intact animal in the case of mice, guinea pigs, rabbits and dogs. The crystalline preparation of hematoporphyrin hydrochloride was weighed out for the proper dosage and then rubbed up with a small quantity of a one per cent solution of sodium bicarbonate and diluted with water.

---

continued until the acid was saturated. The hydrogen bromide gas was prepared by dropping bromine on a mixture of red phosphorus and water. The gas was purified from the free bromine by passage through a U-tube containing red phosphorus spread upon glass beads. Approximately 12½ grams of phosphorus, 25 grams of water and 125 grams of bromine were used for each 100 cc. of glacial acetic acid.

Only enough bicarbonate was used to obtain a clear solution, the liquid being faintly alkaline in reaction. This solution was filtered and injected either subcutaneously or intravenously according to the size of the animals.

The results in the case of the mice repeated those already carefully described by Hausmann. In the dark no effect was observed. In sunlight or under a 400 watt Mazda lamp the animal showed all the symptoms described by Hausmann, ending, when the dose was sufficient, 2 to 3 mgm., in coma and death.

In rabbits and guinea pigs the dose given was usually 50 mgm. per kilogram of animal. The symptoms exhibited were essentially the same as in the case of the mouse. The rabbits were utilized to make examinations of the blood for the pH and for sugar. The pH determinations, made according to the method described by Levy, Rowntree and Marriott, (1915) and by Hawkins (1923) showed slight variations that were not consistent and were within the range of experimental error. The sugar determinations made according to the method of Maclean (9) showed no consistent change after the injection of hematoporphyrin and exposure to the light. Similar negative results are reported also by Smetana (1927).

Experiments on dogs. Most of the experiments on dogs were done upon anesthetized animals prepared for observations upon blood pressure, pulse rate, respirations, etc., but as a preliminary test observations were made upon two intact animals. Comparable amounts of hematoporphyrin (58 mgm. per kgm. of body weight) were dissolved in a 1 per cent solution of sodium bicarbonate and injected into the femoral vein. This was done under aseptic conditions and with local anesthesia. One animal was then placed in the dark and the other was exposed to the sunlight. The former showed no symptoms at all during the 48 hours in which he was kept in the dark. He ate well and was playful. On the third day, when brought into the light he showed very slight discomfort; this condition, however, had passed entirely by the fourth day. This dog was kept three weeks in a light-room, during which time he was well and playful. The dog which was given hematoporphyrin and placed immediately in the sunlight showed marked symptoms. There was first a general erythema of the skin, followed soon by an intense cutaneous irritation. The dog, apparently suffering acutely, scratched and bit at the skin, and was in constant restless movement. He had paroxysms of howling and rapid breathing, and made attempts to avoid the light. Eventually the skin became cyanotic and edematous. On the second morning, after a night in the dark, he was quiet but lethargic and unresponsive. Respirations were slow and the skin was cyanotic in appearance. On placing him in the sunlight the paroxysms of howling and rapid breathing reappeared. The dog was removed on the third day to a poorly lighted room in the hope that recovery might take place. This, however, did not occur. Within several days

necrotic areas appeared in the parts of the skin which had been most exposed to the light. By the eighth day his condition was so bad that it was thought best to chloroform him. Autopsy showed clean normal viscera. There were no hemorrhagic areas on the small or large intestine. The kidneys appeared normal, with the exception of a slight reddening in the medullary portion. No other abnormalities were noted in these organs. The brain was normal.

The effect upon blood pressure, pulse rate and respiration. In these experiments the animal was anesthetized throughout the experiments by means of an ether bottle connected with the trachea. Blood-pressure tracings were taken from the femoral artery and the respiratory movements were recorded. The hematoporphyrin, 60 mgm. per kgm. of body weight, was dissolved in saline, 0.9 per cent solution of sodium chloride, after previous treatment with a 1 per cent solution of sodium bicarbonate, care being taken that the resulting solution was only slightly alkaline. This solution, after filtration, was injected slowly into the femoral vein. For the light experiments dogs were selected with little hair over the abdominal surface and, the animal being on his back, the whole of this surface was exposed to the light of a 400 watt lamp suspended above at a sufficient distance to prevent undue heating. In the dark experiments the animal was covered completely with a light-proof wrapping immediately after the intravenous injection of the hematoporphyrin. The results obtained were quite consistent and are illustrated in figures 1 and 2 which give the records obtained in two cases.

The first effect of the injection of hematoporphyrin into the circulation was a temporary fall in the blood pressure. This was observed in the animals which were kept in the dark after injection, as well as in those exposed to the light. The true effect of hematoporphyrin and light, as shown in figure 1, came on about one-half hour after injection. It was characterized by a rise in blood pressure, paroxysms of hyperpnea, and by the appearance of an intensely high pulse rate. At this time a marked erythema was present over the entire body. After several hours the blood pressure began to fall and sank more or less rapidly to a lethal point. As the pressure began to fall the skin became cyanotic in appearance and somewhat edematous. The pulse and respirations continued very high until just before death. The collapse in blood-pressure appeared to be the immediate cause of the death of the animal.

The symptoms were quite different when an animal was kept in the dark after injection of hematoporphyrin. This is seen in figure 2. There was an initial fall in pressure which was followed within a half-hour by a return to normal pressure. This level was then maintained during the experiment, which was finally discontinued after it had been running seven hours. There was in this case no vasomotor collapse. The rather marked rise in

respiration and pulse rate which came on gradually may be attributed to the long anesthesia.

The immediate cause of death in the light animals was apparently the fall in arterial pressure and in connection with the explanation of the cause of this collapse it seemed important to ascertain whether a similar effect would be obtained if only a small portion of the skin area was exposed to the light. Only a single experiment of this kind was done owing to lack of hematoporphyrin, but the results seemed to be decisive. They are

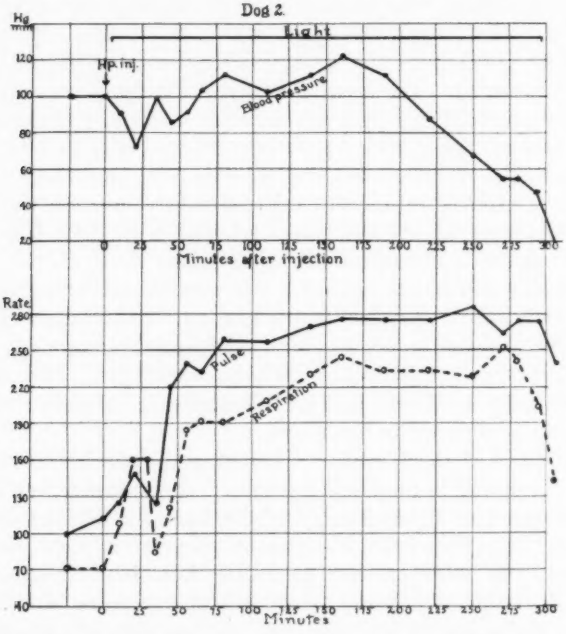


Fig. 1. Chart showing the blood-pressure, pulse and respiration in an hematoporphyrinized dog exposed to light.

shown in figure 3. An area of 9 square inches on the abdomen was exposed to the light, the rest of the animal being protected by a light-proof cloth. The exposure was maintained for seven hours. It will be noted that while there was a rise of pressure, following the initial fall, it came on late, and so long as the experiment lasted there was no indication of a permanent and lethal fall in arterial pressure. It is to be noted also that only the portion of the skin actually exposed to the light showed the characteristic cyanotic coloring at the end of the experiment. In those cases in which the whole of the front of the animal was flooded with light the terminal and fatal fall in pressure made its appearance in about three hours.

The results of these experiments show that in the light animals there was an excitation of the medullary centers, just as the observations on intact animals exposed to hematoporphyrin and light indicate that, in the initial stages, there is a high degree of excitation of the central nervous system. The question arises whether this excitation is of a reflex character due to cutaneous irritation, or whether, possibly, it is due to some toxic irritant formed in the skin and carried to the nervous system and other parts of the body by means of the circulation. To throw some light upon this point a series of experiments was made upon the isolated heart of the terrapin.

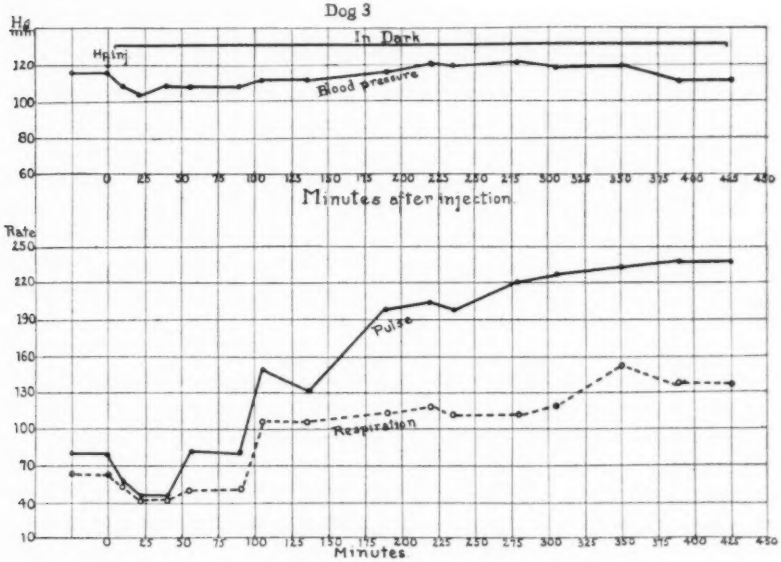


Fig. 2. Chart showing the blood-pressure, pulse and respiration in an hematoporphyrinized dog kept in the dark.

Experiments upon the heart of the terrapin. After destroying the brain and pithing the spinal cord the plastron was removed and the heart exposed. A ligature was placed round the frenum which served as the point of pull for a kymograph record of the heart's contractions. The veins leading into the sinus were ligated with the exception of the left superior cava which served as the path of inflow of the circulating liquid. A ligature under all the arterial branches at the base of the ventricle served to tie in the outflow cannula, which had been placed in one of the arteries, and at the same time to close off the other branches. The circulating liquid was supplied from two Mariotte's flasks which were used alternately

so as to maintain an uninterrupted circulation at a constant low pressure. The terrapin's heart when isolated in this way and supplied with a Ringer's mixture of proper composition will beat well and regularly for many hours. As a control for subsequent experiments it was shown that when such a heart is illuminated by the light from a 400 watt lamp, so placed as not to cause undue heating, the beats are not affected.

When hematoporphyrin was added to the Ringer's mixture to a concentration of 0.006 per cent, and the heart was carefully protected from the light by suitable screens, no injurious effect could be observed. There was often some change in rhythm when the hematoporphyrin solution was

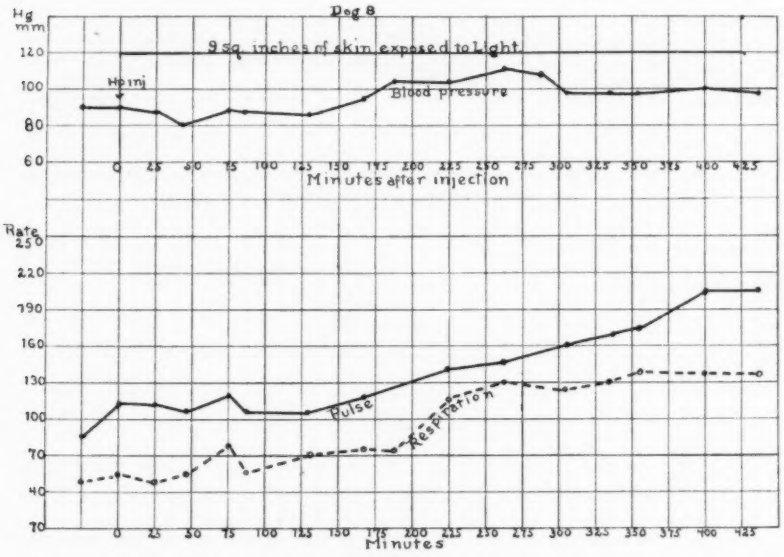


Fig. 3. Chart showing the blood-pressure, pulse and respiration of an hematoporphyrinized dog in which a small area of the skin was exposed to the light.

turned on, owing probably to a difference in reaction, but the heart soon settled down and beat regularly throughout the time of the experiment which was usually four hours. When, however, the heart was exposed to the light of the 400 watt bulb while the hematoporphyrinized Ringer's mixture was circulating through it, the effect was entirely different. Usually within an hour or sooner the heart began to show marked irregularities in rhythm, such as grouped beats with pauses between, the beats became weaker and the ventricle often went into vermicular contractions. After an hour to an hour and a half the ventricle ceased beating. The contractions of one or both auricles might continue for a longer time, but

they also became gradually feebler up to the point of cessation. Both auricles and ventricles, after an initial dilatation, died finally in a state of tonic contraction. If the heart was first circulated with the hematoporphyrinized Ringer's mixture in the dark for half an hour to an hour and the light was then turned on its effect became evident in a few minutes, as though some combination had taken place in the dark between the heart substance and the hematoporphyrin which then reacted promptly to the light when it was turned on. The terrapin's ventricle offered, therefore, a convenient test object for studying some of the conditions controlling the photodynamic action of hematoporphyrin. The following experiments were tried.

1. *The effect of illuminating the hematoporphyrinized solution alone.* This was accomplished by having about 20 feet of thin walled glass tubing arranged in compact coils and inserted on the inflow tube so that the liquid passing through it could be radiated from the lamp, while the heart was kept in the dark, covered by a light-proof cloth. The results showed that the heart was not affected in the least. It beat regularly through the four hours of the experiment. The conclusion from this experiment, therefore, as from similar ones made by other observers upon the red blood corpuscles, is that previous radiation of the hematoporphyrin solution alone does not develop any toxic substance. The sensitizing effect of the hematoporphyrin is exerted only when it is in contact or combination with the tissues.

2. *The effect of circulating liquid from a light-heart through a heart kept in the dark.* In this experiment two terrapin hearts were arranged in tandem so that the Ringer's solution containing hematoporphyrin after passing through one heart, which was exposed to the light, passed on through the second heart, which was kept in the dark. This arrangement was effected by connecting the outflow cannula from the aorta of the first heart with the vena cava of the second heart. Twenty-five minutes after the beginning of the experiment the ventricle of the heart exposed to the light became irregular and soon went into vermicular contractions. After forty-five minutes it ceased beating entirely, going into tonic contraction. The heart in the dark on the contrary gave strong and regular beats for two hours. At the end of that time the protecting cover was removed and it also was exposed to the light. Within fifteen minutes its contractions became irregular and in thirty-five minutes it had ceased to beat. It seems to be evident from this experiment that whatever may be the nature of the toxic action exerted upon a tissue exposed simultaneously to hematoporphyrin and light there is probably no soluble toxic material formed and given off to the circulating liquid so as to affect a distant organ. In the hematoporphyrinized intact animal the evidences of excitation of the central nervous system are probably not due to stimulating substances formed in the skin,

where the light is acting, and carried by the blood to the nerve centers. The irritation must take place in the skin itself where the combined action of the light and hematoporphyrin occurs, and the effect upon the central nervous system is probably reflex, due to stimulation of cutaneous receptors.

3. *The fixation of hematoporphyrin in the tissues.* From statements made above it is probable that the tissues have the property of combining with or fixating the hematoporphyrin, and that it is this combination which is affected by the light. This conclusion is strengthened by an experiment in which a terrapin's heart kept in the dark was perfused for an hour and a half with a Ringer's mixture containing hematoporphyrin in the usual concentration (0.006 per cent). At the end of this time the heart was beating well. It was then perfused for 15 minutes with a Ringer's mixture containing no hematoporphyrin. The effluent was not returned to the flask so that the heart was thoroughly irrigated during this period, and all traces of the hematoporphyrin solution were removed. After fifteen minutes and while circulation with the Ringer's mixture was continued the cover was removed and the heart was exposed to the light. Almost immediately the heart became irregular, vermicular contractions appeared and in six minutes the heart was dead. A similar experiment is reported by Günther (1912) upon red corpuscles. When exposed for 2 hours in the dark to hematoporphyrin and then washed six times to remove the hematoporphyrin they were still found to be sensitive to light.

One may conclude from such experiments that an unstable compound of some kind is formed slowly between the tissues and the hematoporphyrin. As long as light is excluded no injury to the tissue results, but when light falls upon the tissue some change is effected that brings about its death.

4. *The protective action of blood serum.* In these experiments the circulating medium consisted of ox's serum diluted with two volumes of 0.9 per cent solution of sodium chloride. Hematoporphyrin was added to this mixture in the usual concentration and it was perfused through the isolated terrapin-heart while exposed to the light of a 400 watt lamp. The usual result of the action of the light was not obtained. The heart continued to beat regularly for as much as four hours, although apparently not so vigorously nor so normally as would have been the case in the absence of hematoporphyrin. These experiments indicate that something in the serum protects the heart tissue from the photodynamic action of hematoporphyrin—a result which has been obtained by other workers in the case of the red blood corpuscles (Busck, 1906). A systematic effort was made to determine which constituent of the serum exerts this protective action. In the series of experiments carried out it was not always possible to interpret satisfactorily the results obtained, since it is evident that, owing to individual differences in the hearts used, a partial protection might escape detection. But the results obtained seemed to justify the following conclusions.

a. The protective substance is not ether soluble. A serum which was thoroughly shaken out with ether still exhibited a distinct protective action.

b. The protective substance is probably one or more of the blood proteins. The proteins were removed from the serum in great part by acidulating with acetic acid and heating on the water bath. The precipitate was filtered off and the filtrate was shaken with ether which was then removed by a separating funnel. The ether in solution in the serum was removed by heating and during this process more protein separated out. The final filtrate was clear. It was brought to a slightly alkaline reaction, hematoporphyrin was added in the usual concentration and it was then perfused through the terrapin-heart while exposed to light. The heart soon became irregular and within sixty minutes had ceased to beat.

c. Euglobulin and pseudoglobulin have no protective action. The euglobulin was obtained from ox-serum by saturation to 33 per cent with ammonium sulphate. The precipitate was filtered off, washed with a solution of ammonium sulphate one-third saturated, and was then dissolved in water and freed from ammonium sulphate by dialyzing in a collodion sac, first against running tap water and finally against distilled water until the dialysate gave no reaction with barium chloride. The euglobulin was brought into solution in a Ringer's mixture forming approximately a one per cent solution. Hematoporphyrin was added in the usual concentration and the solution was tested upon the terrapin-heart, both in the dark and in the light. In the dark the heart beat well for the period of the experiment, 2 hours. In the light the heart became irregular in twenty minutes and after thirty minutes ceased beating.

In the serum from which euglobulin had been removed the pseudoglobulin was precipitated by the further addition of ammonium sulphate to 50 per cent saturation. This precipitate was washed and dialyzed to remove all traces of the ammonium sulphate and was then brought into solution in a Ringer's mixture containing the usual amount of hematoporphyrin. This solution contained approximately 3 per cent of pseudoglobulin. When circulated through a heart kept in the dark there was no injurious effect, the heart gave a regular beat. When the light was turned on tone waves appeared, the amplitude of the contractions decreased and after thirty minutes evidences of incoördination were seen. The heart ceased beating after one hour and thirty minutes. While it is possible that the pseudoglobulin gave some protection it is evident that it was much less effective than that shown by the whole serum.

These two results were supported by an additional experiment in which the euglobulin and pseudoglobulin were salted out together and their effect tried upon the heart in combination with hematoporphyrin and light. The photodynamic effect of the hematoporphyrin appeared although somewhat delayed. The heart ceased contracting after two hours.

d. Serum albumin has a protective action. Serum albumin when isolated from ox serum has a marked protective action. The serum albumin was prepared by two methods. First by salting out euglobulin and pseudoglobulin by adding ammonium sulphate to fifty per cent saturation, and then adding ammonium sulphate to the filtrate to further saturation. The final precipitate was washed and dialyzed as described above and then tested upon the heart in a three per cent solution in a Ringer mixture. In the second place serum albumin was prepared in crystalline form according to Hopkins' method and used upon the heart in a three per cent solution. In both cases when hematoporphyrin was added and the heart was exposed to light it continued to beat regularly for four to five hours. The serum albumin seems to confer as complete protection as was obtained from the whole serum.

e. Protection is not conferred by egg albumin or peptone. Crystallized egg albumin prepared by Hopkins' method and used in a concentration of 1.3 per cent, Merck's egg albumin in 2 per cent solution, and Witte's peptone in 1.5 per cent solution were all tested upon the heart in combination with the hematoporphyrin and light. None of them exhibited any distinct protective action. Evidences of photodynamic effects appeared within 15 to 20 minutes and the heart ceased beating entirely after perfusion for 1 to 1.5 hours. In his experiments upon red corpuscles Busck, making use of eosin as a photobiological sensitizer, states that solutions of egg white exert an inhibitory effect, although in a footnote he adds that its protective influences in many cases was not clearly marked. We made use of the egg albumin separated from the white of eggs and in our experiments this gave no distinct evidence of protective action in the concentrations used.

Discussion. The fatal effect of hematoporphyrin upon animals exposed to light is a result for which an entirely satisfactory explanation has not been given, but certain facts have been brought out in these experiments which throw some light upon the problem. It is evident from previous work upon hemolysis and from the experiments upon the turtle's heart recorded in this paper that the hematoporphyrin must be in direct contact with the tissue in order for the light to exert its injurious action. Radiation of the solution of hematoporphyrin before it comes into contact with the heart muscle or the red corpuscles is without effect. If the hematoporphyrin is in contact with the tissue, the light causes, first, a stimulating effect, followed later by a paralysis of the tissue cells and then by their death. That there is first a stimulating effect upon the cutaneous sensory nerves is shown in mammals by the intense itching or pain which develops, and which seems to give the animal such marked distress. The cause of the initial erythema over the illuminated area has not been demonstrated, but it is due possibly to a loss of tone in the arterioles. The dusky cyanotic color

that appears much later in these areas and is coincident, as shown by the experiments on dogs, with a fatal fall in blood pressure, seems to be caused by the complete paralysis of the whole vascular bed affected, leading to a stagnation of the blood flow. This effect may be seen directly in the frog's web when observed under the microscope after the injection of hematoporphyrin.

In discussing the lethal action of hematoporphyrin, the primary question that arises is whether the effect of the light is entirely local or whether it produces possibly some toxic substance in the injured skin which is carried off in the blood to stimulate and then to paralyze the central nervous system. The latter hypothesis might be used to explain the symptoms observed, but it is lacking in direct experimental evidence. So far as our work goes, the results are opposed to this theory. When two turtles' hearts were connected in tandem, so that the circulating liquid containing hematoporphyrin flowed first through one and then through the other, it was found that when the first heart was exposed to a bright light, the second heart, which was in the dark, was not affected in the least, although the first heart soon succumbed to the effect of the light. One might suppose, of course, that the hypothetical toxic substance was formed in minute quantities and became too highly diluted in the circulating liquid to affect the second heart, or possibly that it was quickly destroyed when carried away from its place of origin. But these assumptions are somewhat gratuitous in the absence of experimental proofs. The direct and more probable conclusion is that there is no formation of soluble toxic material, but that at the points illuminated there is a local reaction, resulting first in stimulation and then in paralysis and death. In the experiments made upon hematoporphyrinized dogs exposed to the light, there was first a rise of blood pressure together with an increased respiratory and cardiac rate, but later the blood pressure began to fall somewhat suddenly and declined steadily until the death of the animal. These results might be explained by assuming that the medullary centres were first stimulated and later paralysed, the paralysis of the vasomotor centre causing the fatal vascular collapse. But this hypothesis assumes the formation of a toxic substance in the illuminated skin and its conveyance by the blood to the nerve centres. If the experiments referred to above are accepted as disproving this hypothesis, we must explain the results in some other way. It seems quite possible that if the direct photodynamic action is confined to the skin, the first stimulating effects on blood pressure, respiration, movements, etc., may be referable to reflex excitation of the nerve centres from the sensory nerves of the skin. The later collapse of blood pressure to a lethal point may be due to an increasing paralysis of the cutaneous vascular bed, leading to a stagnation and pooling of the blood in the peripheral circulation, as in the case of shock. That the total effect upon the animal

varies with the extent of skin area exposed to the light is shown by the experiment on a dog in which only a small area (nine square inches) was illuminated. In this case no indication of a fatal fall in blood-pressure developed after an exposure of seven hours. This result is in accordance with the hypothesis suggested above.

One other point may be discussed briefly: A number of observers have shown that the hemolytic action of light and hematoporphyrin upon red corpuscles is prevented by the blood plasma or serum. In our experiments we have demonstrated that the same protective effect of serum is exhibited in the case of the turtle's heart. Schmidt and Norman (1920), accepting the theory suggested by Busck that the protective action is due to the proteins, further suppose that this effect is referable to certain special amino acids in the proteins, especially to tyrosine and tryptophane. This explanation is not borne out by our results upon the turtle's heart. The analysis of this protective action given in our experiments indicates that it is not exhibited by all the proteins of the serum, but is confined largely or entirely to serum albumin. Euglobulin, pseudoglobulin, egg albumin and peptone, which contain tyrosine, possess little or no protective action. What it is in the serum albumin which confers upon it this special protective effect, cannot be stated. It is possible, of course, that it may be some unknown substance which adheres to the albumin when precipitated by ammonium sulphate, but the fact that the albumin when purified by crystallization, continues to exhibit this property in a marked way is an argument against such an explanation.

The protective influence of the blood plasma makes it improbable that the death of hematoporphyrinized animals is due to an hemolysis of the red blood cells. Hausmann found no evidence of hemolysis in mice killed by hematoporphyrin, and Meyer-Betz got negative results from examination of his own blood.

SUMMARY OF RESULTS

1. The fatal effects of light on hematoporphyrinized animals were observed upon frogs, mice, guinea pigs, rabbits and dogs. While the external symptoms varied somewhat in the different mammals, they followed the same general course: First, evidence of marked cutaneous irritation together with great motor excitation, erythema and edema of the skin and hyperpnea, followed eventually by cutaneous cyanosis, loss of sensibility, stupor and death. In animals that survived necrotic areas developed on parts of the skin exposed to the light.

2. The isolated turtle's heart, when perfused with a Ringer's solution containing hematoporphyrin and exposed to light, develops an incoordinated beat and soon dies in a contracted state.

3. Illumination of the hematoporphyrin solution alone has no injurious effect if the heart is kept in the dark.

4. Experiments upon two hearts perfused in tandem, with one heart in the light and one in the dark, showed that the toxic action of the light is localized. No evidence was obtained of a soluble toxic substance carried off in the circulating medium.

5. Blood serum protects the hematoporphyrinized heart from the injurious effects of light. It is shown that this protective action is due to serum albumin. The globulins exert little or no protection.

6. Kymographic observations on dogs showed that after an initial fall caused by the injection of hematoporphyrin there is a rise of blood pressure beyond normal. After a certain time the pressure sinks slowly but steadily to a lethal level. There is a marked acceleration of the respirations and pulse rate, lasting beyond the point of vascular collapse.

7. The fatal effect upon dogs is referable directly to the fall in blood pressure. It is concluded that the vascular collapse is not due to toxic substances formed in the skin and carried to the nerve centres, but to paralysis of the cutaneous circulation.

8. The photodynamic action of hematoporphyrin is limited to the parts directly exposed to light. There is some evidence that the hematoporphyrin combines slowly with a substance or substances in the tissues, and that this compound is formed in the dark as well as the light. It is the action of light on this combination which gives rise to the injurious effects. The general symptoms exhibited by the animal may be explained as due to primary stimulation followed by paralysis or death in the structures acted upon by the light.

9. No significant change in blood sugar or in the blood reaction (pH) was detected in animals exposed to hematoporphyrin and light.

BIBLIOGRAPHY

Busck. 1906. Biochem. Zeitschr., 1, 425.
Günther. 1912. Deutsch. Arch. f. Klin. Med., cv, 89.
Hausmann. 1908. Biochem. Zeitschr., xiv, 275.
1911. Ibid., xxx, 276.
1914. Ibid., lxvii, 309.
Hawkins. 1923. Journ. Biol. Chem., lvii, 493.
Levy, Rowntree and Marriott. 1915. Arch. Int. Med., xvi, 389.
Maclean. 1919. Biochem. Journ., xiii, 135.
Meyer-Betz. 1913. Deutsch. Arch. f. Klin. Med., cxii, 476.
Mörner. 1896. From Küster: Zeitschr. f. physiol. Chem., xxix, 187.
Nencki and Zaleski. 1900. Zeitschr. f. physiol. Chem., xxx, 423.
Pfeiffer. 1911. Abderhalden: Handb. d. biochem. Arbeitsmethode, v[1], 563.
Raab. 1900. Zeitschr. f. Biol., xxxix, 524.
Schmidt and Norman. 1920. Journ. Inf. Dis., xxvii, 40.
Smetana. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 821.

CALCIUM AND ULTRA VIOLET IRRADIATION

A. The Effect of Ultra Violet Radiation upon Serum Calcium B. Calcium Utilization on a Calcium-Poor Diet with Ultra Violet Radiation

LAWRENCE T. FAIRHALL

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication December 21, 1927

The effect of ultra violet radiation upon calcium utilization has, for several years, occupied the attention of many investigators because of the pronounced effect of this form of energy in the treatment of rachitic disturbances. The fact that irradiation, in the presence of an adequate diet, should increase the deposition of calcium in the bones of rachitic animals is extraordinary. The following work represents further study of the effects of ultra violet radiation upon serum calcium and calcification.

Effect on blood calcium over long periods of time. It has been supposed that irradiation increases the blood calcium although the evidence has been conflicting. In confirmation of this, Grant and Gates (1) found that the serum calcium of rabbits was increased by moderate irradiation, and Mayerson, Gunther and Laurens (2) also found that prolonged irradiation increased the serum calcium content. Howe and Medlar (3), however, found no evidence that ultra violet rays affected the calcium content of serum when the amount was within normal limits before treatment. Woringer (4) found that exposure invariably raised the blood calcium to normal in infants when it was deficient. At the same time all clinical signs of calcium lack disappeared. Rothman and Callenberg (5) found that the serum calcium of hospital patients was increased by prolonged exposure to radiation.

More recently Moritz (6), in a study of the effect of ultra violet radiation upon serum calcium, found that no changes occurred in the total calcium nor in the percentage of diffusible calcium of the blood serum of normal rabbits *in vivo* or *in vitro*, although Clark (7) found the diffusible calcium of blood serum to be increased after the serum was exposed to ultra violet radiation. Denis and Corley (8) found that rabbits exposed daily to ultra violet rays showed no change in blood calcium. Reed and Tweedy (9) found that direct irradiation of blood produced no

immediate change in the blood calcium of dogs under ether anesthesia, while Pincussen (10) found a decrease in blood calcium following irradiation.

Such conflicting evidence leaves one in doubt as to whether an increase in blood calcium is to be expected following irradiation. Since this is an important point to settle from the viewpoint of calcium metabolism, a very careful investigation was made in order to determine *a*, whether significant changes in blood calcium actually occur on exposure of an animal to ultra violet rays, and *b*, whether there is any proportionality between the degree of exposure and change in blood calcium.

Three dogs (two of them large) were selected and their blood repeatedly analysed for calcium before any attempt was made at exposure. Their diet was uniform throughout the course of the investigation. It consisted of cooked meat, bread and dog bread with a small amount of bone meal and the addition of liver three or four times a week. The initial and final weights of the three dogs were, respectively, dog I, 20.4 kgm. and 29.2 kgm.; dog II, 15.5 kgm. and 18.7 kgm.; and dog III, 20.0 kgm. and 23.1 kgm. At the end of the fore-period the dogs were exposed to various amounts of radiant energy and samples of blood withdrawn immediately, ten minutes after exposure, and half an hour after exposure in order to find out whether or not an immediate, transitory rise in blood calcium occurs. Samples of 50 cc. of blood were taken three times a week from each dog over a period of 31 weeks, broken only by a rest period of four weeks. The experiments, therefore, present blood calcium data over a sufficiently long period of time to follow any seasonal variation such as Grant and Gates found to occur with rabbits.

METHOD OF ANALYSIS. Especial care was given to the method of analysis. Generous amounts of serum were used. The serum was weighed into and ashed in platinum dishes and the calcium precipitated as oxalate under the conditions demanded for such small amounts. In the precipitation, the reaction of the solution was controlled with ammonium acetate instead of sodium acetate, owing to the difficulty of completely freeing the filter paper from traces of the latter on washing the calcium oxalate. If sodium acetate is used, traces of this salt are converted to carbonate and oxide on ignition, and give high results. The calcium oxalate was filtered through 5 cm. no. 44 Whatman paper and the calcium determined by a procedure suggested by Dr. C. H. Fiske. The oxalate was converted to oxide in platinum, an excess of accurately standardized 0.1 N HCl added to the oxide, and after solution of the latter was effected, the excess of acid was found by titration against standardized alkali using micro burettes. Preliminary investigation showed this method to be all that could be desired from the standpoint of accuracy.

During the normal period the average calcium content of the serum of dog I was 11.44 mgm./100 gm.; that of dog II 11.73 mgm./100 gm.; and of dog III 11.15 mgm./100 gm. Following this the dogs were exposed at first for five minutes and later for ten minute periods to ultra violet rays. The dogs were irradiated three times a week for periods varying from five to twenty minutes during the course of the investigation. For exposure the dog was strapped to a dog board and the belly and chest exposed at a measured distance. Although the animals were not shaved, a marked erythema was produced at first. The animals soon tolerated large doses, however, without showing much erythema. During the initial radiation period the calcium content of the serum of dog I somewhat slowly increased, that of dog II rose to 16.85 mgm./100 gm. toward the end of the period, while dog III was not appreciably affected. However, a rise in calcium value in the case of both dog I and dog III occurred in the normal period following and reached values of 16.38 and 16.95 mgm./100 gm. respectively. There was, therefore, an average maximum increase in blood calcium amounting to 46 per cent. The interesting feature of this was that the increase was not an immediate response but rather a very slow rise in the case of two of the dogs. Ultra violet radiation, therefore, does not produce the same sort of stimulation as that produced by the parathyroid hormone. In fact, it appears from figure 1 (dogs I and III) that the rise in serum calcium occurs toward the end of exposure rather than during early exposure. A somewhat higher serum calcium level was maintained throughout the normal period following this period of irradiation. Ultra violet radiation induces a higher level of calcium absorption and, as shown later, a somewhat higher degree of utilization on a low calcium diet. It is possible, therefore, that the system finds itself flooded with calcium at a greater rate than it can be utilized.

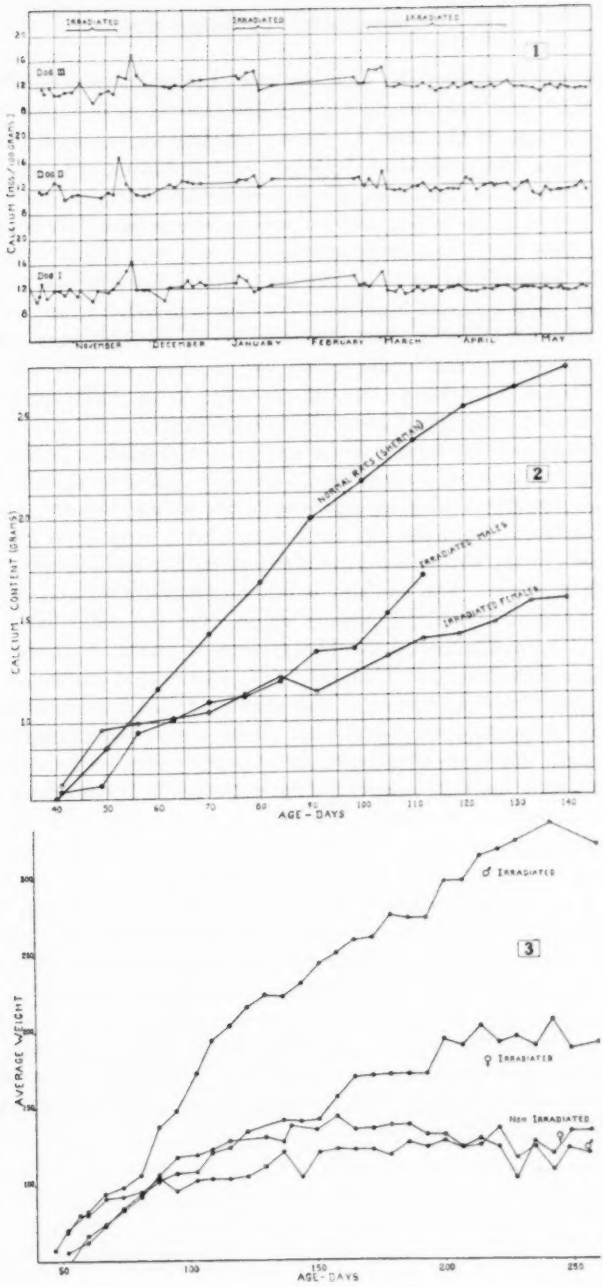
Later periods of exposure produced slight changes but nothing as great as those of the first period. During the second period of exposure the calcium levels were maintained at an average of 12.50, 12.98 and 12.99 mgm./100 gm. respectively, or a general average of 12.82 compared with the preceding normal figure of 11.43—an increase of only 12.1 per cent.

A final long period of exposure to ultra violet rays at twice the former dosage failed to produce any observed change in serum calcium beyond a small initial increase,—in fact, the calcium content of dog I was surprisingly constant over the entire remaining period. This result was wholly unlooked for in view of the previous findings. There was a notable

Fig. 1. Curves showing changes in the calcium content of serum of dogs following irradiation.

Fig. 2. The effect of ultra violet irradiation upon the rate of calcification.

Fig. 3. Growth curves of irradiated and non-irradiated rats on the same diet.


IRRADIATED
IRRADIATED
IRRADIATED
1
Dog III
Dog II
Dog I
CALCIUM (mg/100 cm³)
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
MAY
2
NORMAL RATS (SHERMAN)
IRRADIATED MALES
IRRADIATED FEMALES
CALCIUM CONTENT (GRAMS)
AGE - DAYS
3
♂ IRRADIATED
♀ IRRADIATED
NON IRRADIATED
AVERAGE WEIGHT
AGE - DAYS

Figs. 1 to 3

lack of erythema during the final exposure period in spite of the heavy dosage. This was not due to a great decrease in ultra violet radiation owing to the age of the lamp as shown by measurements of ultra violet intensity using the method of Anderson and Robinson (11), and by the fact that at the conclusion of the experiments other dogs showed considerable erythema following shorter periods of exposure. It is apparent, therefore, that the three experimental animals established tolerance to exposure. These facts may account for many of the irreconcilable statements that appear in the literature. On the one hand serum calcium is not immediately affected after exposure to ultra violet rays but is increased somewhat after lengthy treatment, while, on the other hand, animals reach a point where they are immune to the influence of further radiation as far as serum calcium is concerned.

An alternative explanation of the rise in serum calcium is that it may increase as a result of hemorrhage. Drinker (12) has shown that the amount of cancellous tissue in the long bones varies with the amount of blood formation. Animals that have been bled a number of times show a diminution of the cancellous bone and a relatively smooth-walled cavity filled with marrow. The calcium is obviously absorbed and could conceivably influence the blood calcium value. On the other hand, Milroy (13) has noted a diminished alkaline reserve of plasma following hemorrhage which Henderson and Haggard (14) attribute to diffusion of base from the blood into the tissues. Recently Kerr (15) has investigated the changes in inorganic composition of serum following hemorrhage and found a slight decrease in the sodium content. The calcium content of serum remained normal, however, even after severe hemorrhage. Until further investigation is made, therefore, it seems justifiable to assume that whatever increases in serum calcium were noted in the experiments above were due to the effect of ultra violet radiation.

Effect of ultra violet radiation upon calcification. Coincident with the examination of changes in blood calcium an investigation was made of the degree of calcification that occurs at various ages when an animal is given a low calcium diet, but abundant ultra violet radiation. Young white rats (male and female) of the same age were given food which was low in calcium and consisted of a mixture of rolled oats, corn meal, wheat flour, dextrin, and dried and powdered liver which had previously been well washed and extracted with distilled water. These materials were used in the following proportion:

	per cent
Wheat flour	35.3
Corn meal	23.5
Rolled oats	23.6
Dextrin	11.7
Liver powder	5.9

The salt ration was added to the food in the form of a solution in distilled water. It had the following composition per liter of solution:

	grams
Sodium chloride	47.7
Potassium phosphate (di-basic)	48.7
Magnesium chloride	5.0
Ferric chloride	0.3
Potassium citrate	45.0

The food mixture, made into a dough with distilled water containing a sufficient amount of the inorganic salts (62 cc. per kilo of dry food for a rat weighing 70 grams), was baked into biscuit on an electric hot-plate. Rats, when placed on a diet of these biscuits supplemented with the necessary vitamins, fare rather indifferently. Their growth is very slow and they remain undersized, obviously undernourished rats. Rats grow perfectly normally, however, when this diet is supplemented with calcium to the extent of 10 grams of calcium phosphate per kilo of dry food.

TABLE 1

Comparison of the average calcium content of rats of the same age

	MALE			FEMALE		
	Weight at death	Total calcium	Per cent calcium	Weight at death	Total calcium	Per cent calcium
	grams	*grams*	*per cent*	*grams*	*grams*	*per cent*
Irradiated low calcium diet	320	1.935	0.60	187	1.315	0.70
Non-irradiated low calcium diet	112	0.846	0.75	132	1.175	0.89
Normal rats (data from Sherman)	339	3.750	1.11	220	2.770	1.26

The low calcium rats were exposed daily to the ultra violet rays for a period of ten minutes at a fixed distance, and a pair killed every seven days for analysis. The rate at which calcium is laid down is greater than that indicated with unexposed rats, but is much less than the rate of deposition found by Sherman and McLeod (16) for normal rats as may be seen in figure 2. Irradiated male rats of the same age have a calcium content of 71.7 per cent that of normal males, while irradiated females on this diet have but 70 per cent the calcium content of normal females.

The variation in calcification and growth of rats on this same type of diet was strikingly shown in another type of experiment. Two groups of rats were selected and received identical food and treatment, except that one group was kept wholly in the dark while the other received an exposure of ten minutes daily to ultra violet rays. The first group consisted of six males and eight females, while the second, i.e., those kept in the dark originally, consisted of seven males and eight females. The

growth curves (fig. 3), representing the average weight of each group week by week, show a great difference between the two series. The surviving male rats of those kept in the dark had an average weight of only 112 grams as against 320 grams, the average weight of the exposed male rats. The females fared better, having an average weight of 132 grams as against 187 grams for the irradiated females. In spite of the enormously greater weight of the irradiated male rats, however, their average calcium content (table 1) is only 51.6 per cent of the normal calcium content of a rat of that age based on Sherman's data (16). Similarly, the calcium content of irradiated female rats is but 47.5 per cent of the normal. Nevertheless, irradiated rats, male and female, were able to utilize what calcium was in their diet better than those that lacked ultra violet radiation. The latter males had a calcium content of but 22.5 per cent and the females 42.4 per cent of the normal. It is of interest to observe that the female rats were able to tolerate the

TABLE 2

Calcium content of irradiated adult rats on a normal calcium diet

AGE	WEIGHT AT DEATH	TOTAL CALCIUM	PER CENT CALCIUM
days	*grams*	*grams*	*per cent*
386	423	4.445	1.05
386	363	4.126	1.14
386	387	4.235	1.09
386	386	3.825	0.99
386	347	3.665	1.06
Average.....	381	4.059	1.06

unusual condition of both lack of calcium and of ultra violet radiation much better than the males.

It is significant that of the male and female non-irradiated rats, 50 per cent died before the completion of the experiment, while both male and female irradiated rats all survived and were, to all appearances, healthy, normal, well-grown animals to the end.

This demonstrates rather conclusively that while an animal may be maintained in a healthy condition even on a diet deficient in calcium, providing it is given a sufficient dosage of ultra violet rays, yet this alone is not sufficient to bring his calcium content up to normal. The diet itself must be adequate from the point of view of calcium. The investigations of Sherman and McLeod (16) are of interest in this connection. These investigators found that the addition of fat-soluble vitamins to a diet deficient in calcium did not aid in the deposition of calcium, but when calcium lactate was added to the same diet there was an in-

crease in the calcium content of the animal. Further convincing evidence of this is found in table 2, showing the calcium content of rats, which had been radiated over a period of 250 days, on a normal calcium diet. Their average calcium content is above normal for a rat of the same age.

SUMMARY

Examination of the blood of dogs over an extended period of time indicates that ultra violet radiation slowly causes an increase in serum calcium. This increase is not so marked in subsequent exposure and the animals reach a point where radiation no longer affects the serum calcium.

A study of the degree of calcification at various ages of radiated rats on a low calcium diet indicates a slower rate of deposition of calcium than in the normal rat. While the growth of irradiated rats on a low calcium diet is apparently normal and in marked contrast with that of non-irradiated on the same diet, the average calcium content of such animals is but fifty per cent that of the normal rat of the same age.

On a normal calcium diet, irradiated rats had a somewhat higher calcium content than normal rats of the same age. Both on a low and on a normal calcium diet ultra violet radiation induces a somewhat higher degree of calcium utilization.

BIBLIOGRAPHY

(1) Grant, J. H. and F. L. Gates. Proc. Soc. Exper. Biol. and Med., 1923–24, xxi, 230.
(2) Mayerson, H. S., L. Gunther and H. Laurens. This Journal, 1926, lxxv, 421.
(3) Howe, M. G. and E. M. Medlar. Amer. Rev. Tuberculosis, 1924–25, x, 409.
(4) Woringer, P. Compt. rend. soc. biol., 1923, lxxxviii, 1160.
(5) Rothmann, S. and J. Callenberg. Klin. Wochenschr., 1923, ii, 1751.
(6) Moritz, A. R. Journ. Biol. Chem., 1925, lxiv, 81.
(7) Clark, J. Amer. Journ. Hyg., 1923, iii, 481.
(8) Denis, W. and R. C. Corley. Journ. Biol. Chem., 1925, lxvi, 609.
(9) Reed, C. I. and W. R. Tweedy. This Journal, 1926, lxxvi, 54.
(10) Pincussen, L. Biochem. Zeitschr., 1925, clxi, 61.
(11) Anderson, W. T. and F. W. Robinson. Journ. Amer. Chem. Soc., 1925, xlvii, 718.
(12) Drinker, C. K., K. R. Drinker and C. C. Lund. This Journal, 1922, lxii, 1.
(13) Milroy, T. H. Journ. Physiol., 1917, li, 259.
(14) Henderson, Y. and H. W. Haggard. Journ. Amer. Med. Assoc., 1922, lxxviii, 697.
(15) Kerr, S. E. Journ. Biol. Chem., 1926, lxvii, 689.
(16) Sherman, H. C. and F. L. McLeod. Journ. Biol. Chem., 1925, lxiv, 429.

STUDIES ON THE PHYSIOLOGY OF SLEEP

V. Some Experiments on Puppies

NATHANIEL KLEITMAN

From the Department of Physiology of the University of Chicago

Received for publication December 22, 1927

The tests to be described here were made in 1923, at the same time that similar experiments were performed on human beings. The brains and other parts of the nervous system of experimental and control animals were removed at the moment of death and preserved for detailed histological examination. No one competent to prepare this material was available at that time, and the results of the experiments remained unpublished. The neurological data have recently been obtained.

There were two separate series of experiments. In the first series, a study was made of the effects of artificially produced sleeplessness; in the second, the phenomena accompanying the onset of sleep were observed.

In the first paper of this series (Kleitman, 1923) it was pointed out that all attempts to keep adult dogs awake by Piéron's (1913) method were fruitless. His method consists of chaining the collar fitting around the dog's neck to the top of the cage in such a manner that the dog can stand or sit, but cannot lie down. Falling asleep results in a relaxation of the musculature of the forelimbs. This leaves the animal hanging by the neck, and the choking sensation that results wakes it. By walking the animals at night and leaving them in the cage in the daytime, Piéron was able to keep his dogs awake for several days. Our dogs, however, managed to sleep very well while hanging on the chain, but we found walking to be an excellent method of keeping them awake. We therefore adopted the simple method of watching the animals constantly, tugging them by the chain when they were about to fall asleep, walking them a little when tugging was not effective. With two observers working in 12-hour shifts, we could be sure of the animals remaining awake during the entire period of the test. To avoid the necessity of carrying on an experiment for more than a few days we used puppies whose need for sleep is much more acute than that of adult dogs.

First series. The literature dealing with the effects of prolonged sleeplessness on puppies furnished very meager data on this subject. Manaceine (1894) kept four puppies awake for 4 to 5 days, when they

succumbed to the effects of sleeplessness. These effects consisted of a marked fall in temperature (4 to 5°C. at the point of death) and a decrease of the red blood cell count from the normal five to three or even two million. She later extended her observations using a greater number of puppies (Manaceine, 1897). She reported a diminution in the white blood cell count parallel to that in the red cells; a loss of appetite leading to a fall in weight of from 5 to 13 per cent; peculiar alternate periodic changes in the reflex irritability of the two sides of the body. Deprivation of sleep she found to be fatal in each case, and the younger the puppy the sooner it succumbed. Manaceine also described macroscopic and microscopic changes that she found in the brains and in many other organs of her experimental animals.

Tarchanov (1894) using puppies that were from three weeks to three months old observed that the position of the head relative to the body was an important factor in inducing or preventing the onset of sleep. With the head above the general level of the body some puppies fell asleep more easily than in the horizontal position. Head down, it was impossible to make the animals sleep. Manaceine (1897) thus comments on Tarchanov's observations: "It is evident that the absence of sleep when the head is vertically below the body is explained by stasis of the blood in the vessels of the brain, while the facility of sleep in the opposite position is due to reflux of blood from the head, that is to say, to cerebral anemia."

Tarchanov also observed the exposed brain of puppies in the waking state and during sleep. He noticed that as the animal fell asleep the brain became and remained pale and the superficial blood vessels contracted. Awakening was accompanied by changes of an opposite character.

Method and results. Twelve puppies were employed in the experiments of this series, for the study of experimental insomnia, and parallel observations were made on an equal number of their litter mates. Nearly all the puppies used were born in the laboratory, suckled by their mothers, and were employed for experiments after they were weaned and had grown teeth. They all could drink and feed themselves at the time of the experiment. Their ages varied from six weeks to three months. As a rule two puppies from the same litter, as nearly alike in every respect as could be found were used together, one being kept awake and the other continuing its normal routine as a control. In several cases a daily record was kept of their weight, body temperature, heart and respiratory rates, blood sugar, alkaline reserve of the blood, hemoglobin, and red and white blood cell counts. Each experiment was preceded by period of preliminary observation under normal conditions, of about two weeks' duration. This enabled us to establish a norm for each puppy, the figures obtained (with the exception of the constantly increasing weight) varying but little from day to day. Then one of the puppies was kept awake for a number of days,

at the end of which time it was either killed or allowed to recover. If the puppy was killed or if, as it occasionally happened, it died as a result of the experiment, the control puppy was killed at the time of the death of its experimental litter mate. Autopsies were performed on both puppies, and their brains and spinal cords removed and simultaneously placed in preserving fluid. By this procedure it was possible to ascribe changes observed in the experimental animal, but absent in the control puppy, to the effects of prolonged wakefulness and its attending phenomena.

The period of experimental insomnia varied from two to seven days. As already indicated, the method of keeping a puppy awake consisted of giving a slight pull to the chain attached to its collar when it was about to fall asleep, or of leading it around the laboratory by the chain, if it persisted in falling asleep. After a short walk the puppies generally appeared quite awake for some time, and were allowed to do as they pleased. It was easy to keep the animals awake during the first day of the experiment, but on the second and succeeding days it was progressively harder. From our protocols it appears that it was hardest to keep them awake when they were not walked; easiest, when they were walked frequently and when their attention was attracted to something during the rest periods. Thus bringing in the control litter mate and allowing it to play with the experimental animal often banished somnolence from the latter completely. But after three or four days of continued wakefulness the puppy would lose all interest in its surroundings, and would at times become vicious and unmanageable, biting its litter mate that in its innocence tried to play with it. Some puppies developed a definite photophobia and attempted to get into dark corners, when walked, or into laboratory desks, if the doors of the latter were left half open. But almost to the very last it was possible to bring about a decided change in the animals' behavior by a brisk walk. The transformation of a sleepy apathetic creature into an alert and widely awake animal was very striking at times. The end of the animal's endurance was usually reached when it was too weak to walk. At this time no amount of shaking or pulling by the chain would keep the animal awake. As a rule, the approaching end was characterized by a gradually increasing muscular weakness, the animals standing or walking in a peculiar manner: their front paws would flex anteriorly giving them an appearance of being "flat-footed." At such times the puppies showed disturbances in body righting. If held in the air, back downward, they would let their necks become dorsiflexed, head hanging down (loss of labyrinthine righting reflexes on the head). What is more interesting, they would sleep in that position, if left undisturbed. Some of them slept in the vertical position, head downward, when held in the air by one foot. This condition was never reached before the fourth day, but its development varied greatly in different puppies. At this point the

puppies were allowed to sleep, or else both the experimental and control animals were killed. Those puppies that were permitted to sleep recovered, with the exception of two that died the following day in convulsions, and without waking. Convulsions were occasionally observed in the last days of experimental insomnia in puppies that later recovered from the effects of the experiment, but these may be of a different origin than the above described premortal convulsions. As regards appetite individual puppies behaved differently. Some ate and drank well and kept their weight even after seven days of sleeplessness. Others refused food and sometimes water after 3 to 4 days, and the two puppies that died, although they were allowed to sleep at the end of the experiment, belong

TABLE 1

A comparison between the red blood cell count of a puppy deprived of sleep for several days (W) and that of its litter mate that was allowed to sleep ad libitum (S)

DATE	R.B.C. IN THOUSANDS PER CUBIC MILIMETER		DATE	R.B.C. IN THOUSANDS PER CUBIC MILIMETER	
	W	S		W	S
February 17	4480	4580	March 3	4880	4330
18	*4860*	4640	4	4480	4320
19	*4320*	4480	7	4680	4650
21	*3440*	4640	10	4910	4480
22	*3800*	4520	13	*4816*	4620
25	4220	4590	14	*4724*	4768
26	4460	4640	15	*4100*	4590
28	3928	4860	16	*3920*	4670
			17	*4080*	4672
			18	*3370*	4652
			19	*3520*	4900

The figures in italics indicate blood cell counts made during periods of experimental insomnia.

to this group. The maximum loss in body weight in those puppies that refused to eat was 15 per cent of the original weight (after 5 days of insomnia). There were no appreciable variations in the body temperature of the insomnia puppies. Nor were there any important changes in the heart rate, respiratory rate, blood sugar, alkaline reserve of the blood, hemoglobin, or white blood cells. Minor variations were observed, but similar variations were present in the control puppies, although the two did not always run parallel. For this reason the figures on all the above are not given *in extenso*. The only marked change noted was in the red blood cell count, which fell appreciably, sometimes more than 25 per cent, in the experimental animals. A comparison between the erythrocyte counts of a normal and an insomnia puppy is given in table 1.

The results of a neurological study of the various parts of the central nervous system were entirely negative. Abnormalities could easily be detected in the preparations from the experimental puppies, but similar abnormalities could be seen in the nerve cells of their normal litter mates.

SECOND SERIES. While we were raising puppies for the insomnia tests we observed their behavior, and among other things, their sleeping habits. We were especially interested in the way they fell asleep and woke up. A young puppy will sleep in any situation regardless of the position of its body. So it was possible to remove it from its box-home, handle it, attach recording devices to it, and still have it fall asleep in what is believed to be its natural way of doing so. The outstanding contribution to our knowledge of the behavior of somnolent and sleeping puppies was made by Sidis (1909), who investigated a number of species of animals in this connection. Sidis employed puppies from 2½ weeks to three months of age. By wrapping a cloth around them he immobilized them in the dorsal position, keeping their eyelids closed with his fingers. He found that the puppies struggled and tried to free themselves, but they soon gave up their attempts, remained quiescent and eventually fell asleep. On repetition of the experiment the animals could be put to sleep with greater ease, until after a certain length of time it was no longer necessary to immobilize them by force. From the results of his study Sidis concluded that sleep is brought about by monotony and limitation of movements, and especially by a "diminution in the variability of the volume of sensory impressions."

Method and results. In our own experiments we used puppies of various ages, from a week up. We employed a slightly different method of putting the animals to sleep. Wrapping a cloth around the puppy's body caused it to struggle, sometimes violently, and besides, it hid the abdomen and limbs from view. We therefore placed the puppies in the dorsal position in the "trough" formed by the forearm and the side of the body when the former is held close to the latter. The puppy's head fitted into the hollow of the hand, and the fingers could be closed (if necessary) around the head, or on the eyes. In this way the trunk and limbs of the puppy were entirely free, and it rarely struggled, but would rather nestle against the warm body of the experimenter. After a few minutes in this position the body and limb musculature generally began to relax progressively, and a little later the puppy would be found to be asleep. This was often preceded by a yawn or two. The exact moment of the onset of sleep (if such exists) could not be determined with accuracy. The eyes were kept closed occasionally even when the animals by their movements indicated that they were not asleep. In many cases it was observed that the onset of sleep was preceded by a general trembling or starting movement, involving the legs, but especially the paws. When a tracing of the respiratory movements was taken by means of a small pneumograph tied around the

trunk, the respirations would as a rule become regular, in rate and in amplitude, after such starting movements. Once asleep the puppies could be manipulated considerably without waking them up. They showed a variety of distinct reflexes, the most interesting of which were homolateral and crossed knee-jerks. Their lids could be separated easily to observe the position of the eyes and to test them for the presence of the pupillary reflex to light. The eyes were generally rolled in and down, and the pupillary reflex was present. By dorsiflexing the hand upon the forearm the puppy's head could be lowered considerably and sometimes allowed to hang straight downward without waking the animal. The abdominal musculature was often so relaxed that the abdomen sank in the middle and bulged at the sides. Under these conditions one could easily discern the intestinal movements underneath the abdominal wall. By pinching the abdomen around a distinctly outlined segment of intestine one could lift the intestinal loop, with nothing between the pinching fingers but the mesentery and the double thickness of the abdominal wall.

The length of time that the puppies remained asleep varied considerably in different puppies, and in the same puppy at different trials. As a rule this was only a few minutes. Immediately after a meal the puppies went to sleep very easily and slept longest, but this was rarely more than an hour. When a puppy woke up spontaneously, or was aroused by the observer, it often went through the same trembling movements that characterized the onset of sleep. Yawning was also a frequent accompaniment of awakening. For practical purposes opening of the eyes and coördinated movements of the legs and body were considered criteria of the restored waking state. The most prominent feature of awakening, however, was the stiffening of the abdominal musculature which rendered the anterior abdominal wall taut. Not only was it impossible to pinch off a loop of intestine, one could not see any intestinal movements whatsoever.

Two experiments were performed to detect any possible changes in the appearance of the cerebrum at the onset of sleep and at the moment of waking up. In both cases very young puppies were used (19 and 21 days of age). Each animal was put under ether anesthesia, and parts of the frontal and parietal bones on one side removed to expose the brain. In one puppy the exposed dura was left intact, in the other it was removed. The wound was covered with cotton saturated with warm Ringer solution, and the animal allowed to recover from the anesthetic. For the rest of the day the animal was successively put to sleep, permitted to sleep for a while, and then aroused, and these procedures repeated many times. During the observations the moist cotton was removed from the wound, and the blood vessels of the dura and of the cerebral cortex were watched carefully. The smaller vessels were examined by means of a magnifying glass. No changes whatever, either in the caliber of the blood vessels or in the

general appearance of the brain could be detected when the puppy was passing from wakefulness to sleep, or back from sleep to the waking state. That was also the case when the puppies fell asleep spontaneously.

After a period of prolonged wakefulness it was not necessary to put the puppies to sleep—they would usually fall asleep at once, when allowed to do so. There were no preliminary phenomena to observe in such cases, but when the animals were asleep we attempted to elicit the various reflexes, and as a rule successfully. The main difference between the sleep of such puppies and that induced by permitting normal puppies to relax completely was in the relative ineffectiveness of ordinary stimuli to interrupt the sleep. The insomnia puppies had to be shaken for a long time before they were aroused from the deep slumber into which they plunged at the end of the experiment. If all attempts to wake such an animal were unsuccessful, we considered that it was in a state of coma, and killed it.

Discussion. The behavior of the puppies that were deprived of sleep resembled markedly the behavior of human beings under similar conditions (Kleitman, 1923). That is the most striking of the results obtained, the same ability to stay awake with apparent ease while the muscles were contracting, and the same inability to resist sleep when the muscles of the body were allowed to relax. In their attitude to their litter mates the puppies showed the same gradual change from friendliness to animosity that was observed in human subjects as regards their companions under similar conditions.

Manaceine performed similar tests on puppies, and our results are in agreement with hers in some particulars. For instance, after 3 to 4 days of wakefulness some of our puppies would lose their appetite, and thenceforth their weight would fall. However, there was no lowering of the body temperature in our puppies even after seven days of sleeplessness. Nor was there any change in the leukocyte count with the progression of the experiment. The erythrocyte count did show a definite and marked decrease, but nothing like the 60 per cent decrease reported by Manaceine. She also found that toward the end there was an increase in the number of red blood cell per unit of volume, which she ascribed to thickening of the blood. We found no such increase. Just what the significance of a fall in the red blood cell count might be Manaceine did not suggest in her writings, and it seems to us to be very puzzling. Manaceine also discovered definite changes in the cells of the brain, and in many other organs, as a result of sleeplessness. The brains of our insomnia puppies showed nothing different from the brains of their normal litter mates, but we realize that a negative result here may have been due to some fault in the technique of cutting and staining. For this reason we shall not emphasize these findings.

Piéron (1913) using adult dogs for similar experiments obtained results

that are partly in agreement with those of Manaceine. He found changes in the cells of certain circumscribed areas of the cerebral cortex, but no abnormalities anywhere in the medulla or spinal cord. On the contrary, marked changes in both the medulla and spinal cord are described by Bast, Loevenhart and their associates (1927) who applied the method of experimental insomnia to a study on rabbits. They adopted a method of keeping rabbits awake which consisted of placing them into slowly revolving cages (on a horizontal axis). The rabbits had to move in the direction opposite to that in which the cage rotated and were thus prevented from falling asleep. By this method it was found that after a few days spent in such cages the animals showed definite symptoms of collapse, followed by death. These symptoms in some cases set in as late as the 21st day of the experiment, becoming more pronounced in the days following. As in Manaceine's puppies the temperature fell 4 to 5 degrees, the pulse and respiration were slowed, and finally the animals died in convulsions. The authors believe that by the use of their method "objections raised to previous work in this field will be fully met," and that the effects they observed could be ascribed to "loss of sleep, unattended by any other factor, such as excessive muscular exercise." It is true that there was no excessive muscular work involved in the application of this method (and that is probably why some of the rabbits lived nearly a month in the revolving cage), but there is muscular activity just the same, since the animals must constantly change their position in the cage. In our previous papers (Kleitman, 1923; Lee and Kleitman, 1923) we emphasized the impossibility of divorcing experimental insomnia from muscular activity, and questioned the value of our own results from that standpoint. We were not prepared then (nor are we now) to say to just what extent the effects of experimental insomnia were due to lack of sleep, and to what extent to muscular fatigue. The revolving cage method, keeping the animals in constant activity as it does, without any rest, is more open to objection than many of the methods previously employed, and the results obtained by Bast and his co-workers can by no means be considered as due to lack of sleep only.

Our insomnia animals did not show any marked fall in body temperature, but like Manaceine's puppies some of them refused food and water after 3 to 4 days of sleeplessness. How can this be explained? When collapse and death did ensue, what were they due to? It appears to us that neuromuscular fatigue was greatly responsible for the symptoms exhibited by the puppies after prolonged sleeplessness. Definite muscular weakness and loss of tonus were always in evidence when the animals refused to eat. As long as they had strength to stand and to walk there was no difficulty in keeping them awake. In this connection it may be recalled that one of the subjective symptoms reported by the human subjects that went without sleep for a long time (Kleitman, 1923) was a longing to rest by

sitting or lying down, rather than a desire to sleep. They were not permitted to do that because sitting or lying enabled them to relax their musculature, sleep resulting almost at once. An effective method of keeping animals or human beings awake without making them use their muscles continually has yet to be devised.

Tarchanov's inability to make puppies sleep when their heads were below the level of the body cannot be reconciled with the results of our experiments. Our puppies could sleep when they were held in the air, head downward, if they were sleepy enough. But even normal puppies had no difficulty in falling asleep while lying on an inclined plane, head lowermost. That cerebral anemia is neither the cause nor the result of sleep could be determined by direct inspection of the brain, again in disagreement with Tarchanov's findings.

The results of our experiments in putting puppies to sleep confirm those obtained by Sidis. The outstanding fact here was the tendency of the puppies to fall asleep when the conditions under which they were kept were such that they could relax their body musculature. Sidis emphasized monotony and limitation of movement, but it is possible that these are contributory factors in that they tend to produce a greater muscular relaxation. But really complete muscular relaxation comes on only in sleep. When the puppies were allowed to sleep after a period of several days of wakefulness, their sleeping bodies often appeared entirely formless to the manipulating hand and could be bent or twisted in a most unusual fashion without meeting with any resistance on their part. Likewise, as reported in the above mentioned experiments on rabbits, "After being kept awake for more than a week, it was noted that many of the animals would immediately fall asleep and become perfectly relaxed if removed from the cage and placed upon a level surface."

The starting movement or movements at the onset of sleep were observed by Sidis previously, but their meaning is not clear. Similar movements are executed by some human subjects at the moments of falling asleep, and if they are pronounced enough, they may arouse the somnolent individual.

In the bilateral knee jerk that may be elicited in puppies when they are asleep they differ from humans in whom the knee jerk is either completely abolished or markedly depressed in that condition.

In general the results of these experiments are in support of the working hypothesis presented in the first paper of this series which holds that sleep comes from a decrease in the number of afferent impulses reaching the central nervous system from the sensorium, and that muscular relaxation, decreasing considerably the number of proprioceptive impulses, constitutes the last stage of the process by which sleep is precipitated.

SUMMARY

1. Puppies that were deprived of sleep for several days showed a progressive muscular weakness, sometimes leading to coma and death.

2. The ability of puppies to remain awake for a long time is directly related to their neuromuscular endurance.

3. There is direct and indirect evidence that anemia of the brain is not a necessary condition for sleep in puppies.

4. Sleep in normal puppies is easily induced under conditions that favor muscular relaxation.

The writer is greatly indebted to Drs. C. A. Adams and T. Kreuzer for the assistance they rendered him in the course of this investigation.

BIBLIOGRAPHY

BAST, LOEVENHART ET AL. 1927. This Journal, lxxxii, 121, 127, 131, 140.
KLEITMAN. 1923. This Journal, lxvi, 67.
LEE AND KLEITMAN. 1923. This Journal, lxvii, 141.
MANACEINE. 1894. Arch. Ital. de Biol., xxi, 322.
1897. Sleep. London.
PIÉRON. 1913. Le Problème Physiologique du Sommeil. Paris.
SIDIS. 1909. An experimental study of sleep. Boston.
TARCHANOV. 1894. Arch. Ital. de Biol., xxi, 318.

THE ACTION OF MYRTILLIN IN PANCREATIC INSUFFICIENCY[1]

LEONARD BENJAMIN SHPINER

From the Hull Physiological Laboratory of the University of Chicago

Received for publication January 12, 1928

The experimental use of myrtillin as a therapeutic agent in pancreatic diabetes and diabetes mellitus was presented in Allen's recent paper (1). At his request we have, without prejudice, partly repeated his work and extended it by investigating the action of myrtillin on the hyperglycemia induced in partially pancreatectomized dogs by the administration of desiccated thyroid as recently reported (2).

METHODS. The procedure in these experiments was to put dogs on a daily standard diet of 250 grams beef heart, 100 grams of bread and 250 cc. of milk. This diet, sufficient for their caloric needs, was not changed during the course of the experiment. The dogs were placed in metabolism cages. The total output of urine per 24 hours was recorded, and samples were taken for daily analysis. Toluol was used as a preservative to minimize bacterial decomposition. At the time urine samples were taken, 5 to 6 cc. of blood were drawn from the dog's saphenous vein and put in tubes containing powdered potassium oxalate as an anticoagulant.

Fig. 1. Shows the effect of feeding myrtillin on the blood and urinary sugar curves of a dog which had suffered the loss of $\frac{1}{2}\frac{9}{0}$ of its pancreas (subtotal pancreatectomy).

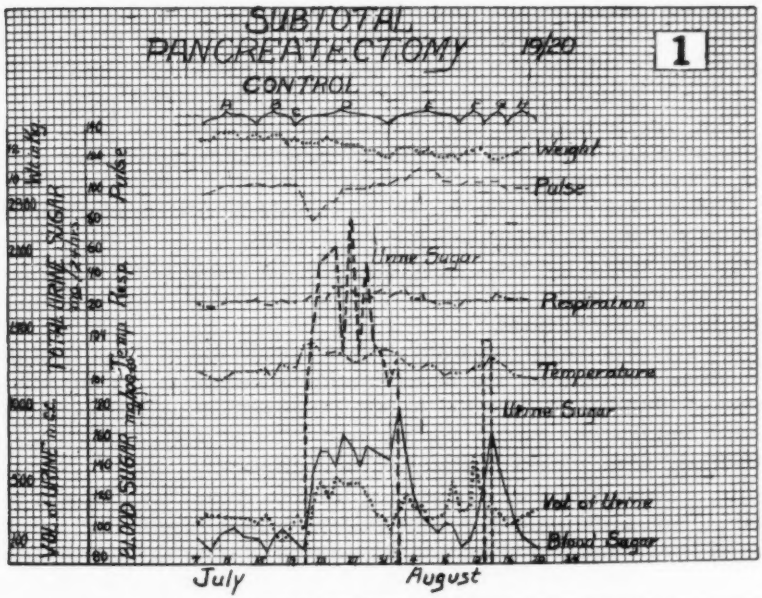
Following a period during which the animal was on a standard diet without, *A*, and with 15 grains myrtillin per day, *B*, the dog was partially pancreatectomized. During *D* the animal received only fluid + 3 (5 grain) tablets of myrtillin daily. The standard diet with 3 (5 grain) tablets daily was resumed during period *E* with sharp drop in urinary and blood sugars. Because of removal of myrtillin during period *F*, the blood and urinary sugars again rose, to decline promptly on addition of 6 tablets (period *G*) and 3 tablets (period *H*) to the standard diet.

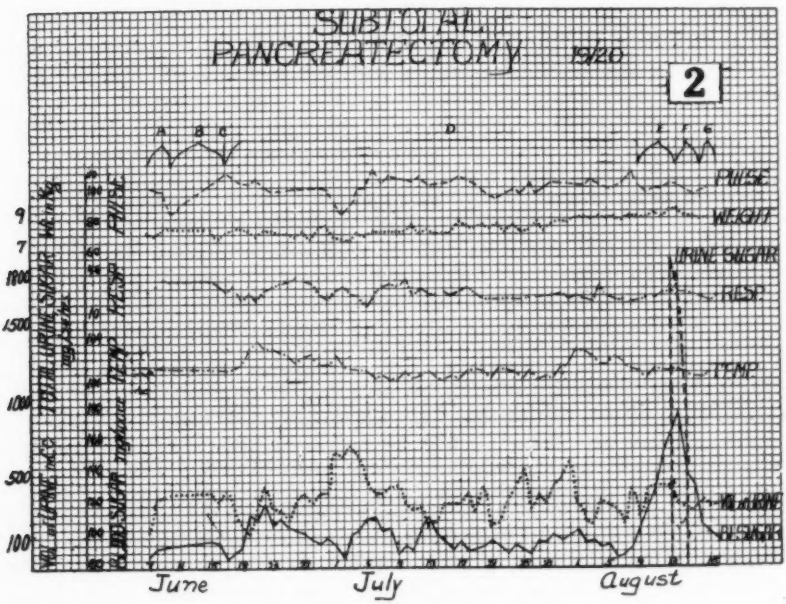
Fig. 2. Shows quite conclusively the action of myrtillin in preventing marked hyperglycemia and glycosuria in a dog following removal of $\frac{1}{2}\frac{9}{0}$ of its pancreas.

Following a period *A* (standard diet) 3 tablets myrtillin were fed daily just before the standard meal (period *B*). Operated at *C* (removal of $\frac{1}{2}\frac{9}{0}$ of its pancreas) the animal was kept on its standard diet + one gram myrtillin daily from June 17 to August 9, 1927 (period *D*). Myrtillin was removed during period *E* followed by a prompt hyperglycemia and glycosuria. Addition of myrtillin during periods *F* (6 tablets daily) and *G* (3 tablets daily) leads promptly to the disappearance of the glycosuria and a return of the blood sugar to a normal level.

---

[1] Aided by a grant from the Frank Billings Medical Club, Chicago, Ill.


SUBTOTAL PANCREATECTOMY 1920
1
CONTROL
Weight
Pulse
Urine Sugar
Respiration
Temperature
Urine Sugar
Vol of Urine
Blood Sugar
July
August


SUBTOTAL PANCREATECTOMY 1920
2
PULSE
WEIGHT
URINE SUGAR
RESP
TEMP
June
July
August

Figs. 1 and 2

To test qualitatively for urinary sugar, Fehling's or Benedict's reagents were used. If the tests were negative, the samples were discarded; if positive, the amount of blood and urinary sugar was determined by the method of Folin-Wu, two determinations being made on each sample.

For blood sugars, the Folin-Wu method was used; two blood sugar determinations were made daily, the average being recorded as the blood sugar level for that day.

Control experiments were run on the animals for three days or upward before preparing them for the initial experiments.

In performing the total pancreatectomy the ducts of Santorini and Wirsung were ligated, and the pancreas was stripped off, care being taken not to leave any remnant.

Two types of partial pancreatectomy were done, one in which $\frac{19}{20}$ of the pancreas was removed, the remnant being connected to the duodenum through the duct of Santorini (subtotal pancreatectomy); the other in which $\frac{7}{8}$ of the pancreas only was removed. The subtotal pancreatectomy was designed to induce a Sandmeyer type of diabetes.

In studying the effect of myrtillin, three to six tablets (5 grains per tablet) were given to the dogs daily, at least one hour *before* feeding time.

To bring about a semblance to a hyperthyroid state, the dosage of 0.5 gram to 0.8 gram per kilo of desiccated thyroid extract as recommended by Kunde (3) was tried and found to have the desired effect. The desiccated thyroid (Armour's) extract was fed by mouth.

RESULTS. I. *The effect of feeding myrtillin to totally pancreatectomized dogs.*

AVERAGE BLOOD SUGAR, MGM./100 CC.		POST OPERATIVE—CONTROL DIET AND MYRTILLIN			
On control diet and myrtillin		Blood sugar		Urine sugar	
Low	High	Low	High	Low	High
81	90	187	220	1.6 gm.	4.5 gm.

MYRTILLIN REMOVED				MYRTILLIN RESTORED TO CONTROL DIET			
Blood sugar		Urine sugar		Blood sugar		Urine sugar	
Low	High	Low	High	Low	High	Low	High
193	248	2.4	6.3	179	215	1.20 gm.	3.2 gm.

Whereas the survival of total pancreatectomized dogs without being given insulin is on the average from 7 to 14 days; in the two totally pancreatectomized dogs kept on myrtillin, the shortest duration of life was 32 days, and the longest was 37 days. The animals were quite lively until a few days before their death. The cachexia which often accompan-

ies diabetes was absent. The dogs were able to maintain their body weight until a few days before their death, when the weight began to drop rapidly, and a terminal cachexia appeared. The downward course to exitus was very rapid. In none of our dogs was there the complete wound healing that Allen (1) described.

II. *Effect of myrtillin on partially depancreatectomized dogs with $\frac{1}{2}\frac{9}{0}$ of the pancreas removed.* See figures 1 and 2.

CONTROL DIET AND MYRTILLIN		POST OPERATIVE—CONTROL DIET + MYRTILLIN			
Blood sugar, mgm./100 cc.		Blood sugar		Urine sugar	
Low	High	Low	High	Low	High
84	94	95	115		

MYRTILLIN REMOVED				MYRTILLIN RESTORED TO CONTROL DIET			
Blood sugar		Urine sugar		Blood sugar		Urine sugar	
Low	High	Low	High	Low	High	Low	High
97	174	No mgm.	1.3 gm.	81	135	Negative (none)	

Removal of $\frac{1}{2}\frac{9}{0}$ of the pancreas leads to diabetes, but in our dogs kept on myrtillin (3 to 6 tablets) diabetes did not develop. The blood sugar was normal and the urine was sugar-free. The wounds healed normally, and the general condition of the dog was good. On removing myrtillin from the control diet, a marked hyperglycemia and glycosuria developed. Feeding myrtillin again reduced the blood sugar to the normal level, the urine remaining sugar free.

In one of our dogs (see fig. 2) kept on myrtillin for a period of 45 days a hyperglycemia and glycosuria developed when the myrtillin was removed. This fact showed that during myrtillin feeding the pancreatic remnant did not hypertrophy, for removal of the myrtillin was followed by a hyperglycemia and glycosuria. It proves conclusively, we think, that the myrtillin prevented the hyperglycemia and the glycosuria. Feeding the dog myrtillin caused a return to a normal state, i.e., absence of glycosuria and a normal blood sugar level.

III. *The effect of myrtillin on the hyperglycemia and glycosuria of potentially diabetic ($\frac{7}{8}$ pancreatectomy) animals during experimental hyperthyroidism.*

CONTROL PERIOD		POST—OPERATIVE, CONTROL DIET + THYROID			
Blood sugar, mgm./100 cc.		Blood sugar		Urine sugar	
Low	High	Low	High	Low	High
83	97	90	158	1.1 gm.	2.4 gm.

MYRTILLIN ADDED TO DIET				THYROID REMOVED FROM CONTROL DIET				MYRTILLIN REMOVED FROM CONTROL DIET			
Blood sugar		Urine sugar		Blood sugar		Urine sugar		Blood sugar		Urine sugar	
Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
114	130			99	112			84	102		

The feeding of desiccated thyroid extract to a pancreatectomized dog ($\frac{7}{8}$ of the gland removed) causes the development of a mild hyperglycemia and glycosuria; with the feeding of myrtillin there is a lowering of the blood sugar to the normal level, and the urine becomes sugar free. Other than this, myrtillin has no effect on the hyperthyroid state of the animal.

CONCLUSIONS

On the basis of these experiments we conclude that 1, myrtillin reduces the hyperglycemia and the glycosuria of a totally depancreatectomized dog (as reported by Wagner and Allen), stabilizing, so to speak, the blood sugar which would otherwise have a tendency to fluctuate within rather wide limits.

2. In dogs which have suffered the loss of $\frac{1}{2}$ of the pancreas the feeding of myrtillin prevents the development of a hyperglycemia and glycosuria.

3. The mild hyperglycemia and glycosuria which follows the administration of desiccated thyroid extract to dogs from whom $\frac{7}{8}$ of the pancreas has been removed is brought to a state of normalcy by the administration of myrtillin.

BIBLIOGRAPHY

(1) ALLEN, F. M. Journ. Amer. Med. Assoc., 1927, lxxxix, 1577.
(2) SHPINER, L. B. This Journal, 1927, lxxxiii, 134.
(3) KUNDE, M. M. This Journal, 1927, lxxxii, 195.

THE PHOSPHATE CONTENT OF RENAL CAPSULAR FLUID IN NECTURUS

FRANCIS O. SCHMITT AND H. L. WHITE

From the Department of Physiology, Washington University School of Medicine, Saint Louis

Received for publication December 29, 1927

In a paper read at the 1925 meeting of the American Physiological Society, White and Schmitt (1926) reported that "Capsular fluid has on every occasion but one failed to give a positive test for phosphate, although there were from 2 to 11 mgm. P per 100 cc. of urine. The test as applied will show phosphate in concentrations as low as between 1 and 2 mgm. P per 100 cc. It is seen that if there is any phosphate in capsular fluid it is present in lower concentration than in the plasma." After carrying out many experiments during the past two years we have come to the belief that our failure to find any phosphate in the few experiments which constituted the basis of our preliminary report on the phosphate question was due to slight variations in the size of the capillary tubes which held the capsular fluid and standard phosphate solutions. In tubes of the size (200–300μ) which we used in our first experiments the blue color produced by Briggs' reagents in a solution containing 1.5 to 2 mgm. P per 100 cc. is barely perceptible and is imperceptible in tubes only slightly smaller. Out of five experiments there was a positive test for phosphate in one. We are at present convinced that the failure to obtain a perceptible color in the other four was due to the use of tubes for capsular fluid which chanced to be slightly smaller than the standard tubes. As a result of eleven subsequent satisfactory experiments in which the size of the tubes was rigidly controlled we are now able to state with certainty that, with a total of fifteen capsular fluids, in fourteen the phosphate content was between 1.3 and 2 mgm. P per 100 cc. and in one experiment no P or less than 1 mgm. per 100 cc., the lowest standard tube employed. The plasma phosphate contents in these experiments ranged from 5.4 to 8.9 mgm. P per 100 cc.

METHODS. To induce anesthesia we have tried immersion in 0.2 to 0.3 per cent paraldehyde for from 20 to 60 minutes, with or without pithing the brain, immersion in 0.1 to 0.3 per cent urethane for similar periods with or without pithing the brain, and in the most recent experiments pithing the brain without an anesthetic. None of these methods gives uniformly satisfactory results; in a considerable proportion of cases the circu-

lation is interfered with. With a poor circulation protein is usually found in the capsular fluid and little or no fluid enters the pipette after that originally in the capsule is withdrawn. Only animals showing an active glomerular circulation are included in this report. In our first experiments the animal was laid on the board, covered with gauze and kept moist by frequent drenching with tap water; later we have employed a board, one end of which is cut down about 1.5 cm. This end is surrounded by a metal wall and the head end of the animal placed in this reservoir, which is filled with tap water; air is constantly bubbled through this. The animals' gills remain in constant motion in the water so long as they are in good condition.

The pipettes used in the later experiments have been from 12 to 20μ outside diameter at the tip, most of them being very close to 15μ, and have been drawn of Pyrex or Jena hard glass. The glass tubes of which the pipettes and capillary receiving tubes were drawn were cleaned by washing first in water, shaking for five minutes in a large test tube containing soap solution, rinsing thoroughly, letting stand overnight in a sulphuric acid-bichromate cleaning solution and then rinsing thoroughly in distilled water. The P determinations made before December, 1926, were by Briggs' method (1922), the later ones by Benedict and Theis' method (1924). The determinations in capsular fluid were carried out by transferring the fluid from the collecting pipette into a glass capillary, adding to this first an amount of molybdic acid reagent equal to one-half the volume of the fluid, then the same volume of hydroquinone—sulphite reagent as of molybdic reagent, drawing the whole volume back into the pipette for mixing, again transferring it to the capillary, sealing both ends with a fine flame and, with the Benedict-Theis method, placing the tube in boiling water for 10 minutes. The standard tubes were prepared as follows. Solutions containing 1, 2, 3, 4 and 6 mgm. P per 100 cc. were made. To two volumes of each of these solutions in a test tube was added one volume each of the molybdic acid and hydroquinone-sulphite reagents. A small amount of each of these mixtures was then drawn into a small glass tube by capillarity, the ends sealed and, with the Briggs' method, allowed to stand for color development; with the Benedict-Theis method the tubes were placed in boiling water for 10 minutes. We have used undiluted standard phosphate solutions corresponding in concentrations to the expected range of undiluted capsular fluid in order to get a deeper blue than when dilutions are made. In a plasma phosphate determination by either method the blue color is read at a volume ten times the volume of molybdic reagent, while our standard tubes and capsular fluids are read at four times the volume of the molybdic reagent. We thus get a deeper blue for our capillary tubes, although the color at this dilution is not quantitatively proportional to the amount of phosphate present. Thus

in making up a set of standard tubes by the Benedict-Theis method 4 cc. of phosphate solution (containing, 1, 2, 3, 4 and 6 mgm. P per 100 cc.), 2 cc. of molybdic acid reagent and 2 cc. of hydroquinone-sulphite reagent are added to the test tubes and mixed; from these the standard capillary tubes are prepared, the boiling taking place after the tubes are sealed. The test tubes, each of which still contains practically 8 cc. of solution, are now boiled for 10 minutes and the solution read in the colorimeter. We then find that 2 mgm. P give twice as much color as 1, 3 mgm. give 1.4 as much color as does 2, 4 mgm. give 1.8 times as much color as do 2, and 6 mgm. give 1.7 times as much color as do 3, or $1.7 \times 1.4 = 2.4$ times as much as 2. This failure of quantitative proportionality makes no difference however, since we have a different standard tube for each concentration; furthermore, the only tubes needed are the 1 and 2 mgm. standards, at which concentrations the color is proportional to the P present. On standing several days in corked test tubes the color increases about 10 per cent as read in the colorimeter. Standard colors in sealed capillary tubes do not change appreciably for several weeks, i.e., a 2 mgm. tube 4 weeks old is not appreciably bluer than a freshly made tube.

With capillaries of the size used, 400μ, the difference in color from any tube to the next is quite marked. One can compare an unknown with the standards nearest it and say that it lies between, let us say the 1 mgm. and the 2 mgm. standards. If it seems to be only slightly less blue than the 2 and definitely bluer than 1 we can call it 1.7 and be certain that we are within 0.2 mgm. of the correct figure. All tubes used for making standards and for receiving capsular fluid had to meet the following requirements: inside diameter at each end to be not less than 380 or more than 420μ and the tube to be of practically uniform diameter throughout its length, i.e., the maximum outside diameter to be not more than 40μ greater than the minimum.

Results. Protocols (table 1) are given only of those experiments where the circulation was good and the experimental procedure technically satisfactory. We have taken the absence of a cloud on adding the molybdic acid reagent as evidence of absense of protein. When the Briggs' molybdic reagent is added to Necturus plasma diluted 1 to 100, in a 400μ tube by the same technique used with capsular fluid, a definite cloud is produced with Necturus plasma diluted 1 to 125 a barely perceptible cloud is formed. With dog's plasma diluted 1 to 300 a marked cloud is produced, at 1 to 350 a definite cloud and at 1 to 400 a barely perceptible cloud. With the Benedict-Theis reagent a definite but not marked cloud is formed in Necturus plasma diluted 1 to 100, no cloud at 1 to 125; this reagent produces a definite cloud in dog's plasma at 1 to 300, a barely perceptible cloud at 1 to 350 and none at 1 to 400. All capsular fluids (except those otherwise specified) reported on in the protocols were protein-free according to these

tests. It will be noted that all but one of the capsular fluids had from 1.3 to 2 mgm. P per 100 cc. although the plasma P content was 3 to 4 times

TABLE 1

1. May 22, 1926. Male. 0.2 per cent urethane. Urine before experiment, 1.6 mgm. P per 100 cc. Glomerular fluid between 1.2 and 2.0 mgm. P per 100 cc., estimated at 1.6. Plasma 6.2 mgm. P per 100 cc.
2. June 10. Female. Glomerular fluid "much lighter than 2 mgm. standard." Called 1.5 mgm. P per 100 cc. No urine. Plasma P not determined.
3. June 14. Female. Fluids from 2 capsules each showed between 1.2 and 2 mgm. P per 100 cc., estimated at 1.7. Bladder urine before experiment 1.6 mgm. P per 100 cc. Plasma P not determined.
4. Dec. 10. Female. 0.3 per cent urethane. Bladder urine before experiment 7.3 mgm. P per 100 cc. Capsule fluid between 1 and 2 mgm. P per 100 cc., estimated at 1.7. Plasma 8.9 mgm. P per 100 cc. Urine accumulating in bladder during experiment had 5.6 mgm. P per 100 cc.
5. Dec. 12. Female. Bladder urine before experiment 2.8 mgm. P per 100 cc. 3 cu. mm. glomerular fluid in one-half hour, had between 1 and 2 mgm. P per 100 cc., estimated at 1.7. Plasma P 6.2 mgm. per 100 cc.
6. Dec. 14. Male. Fluids from 3 capsules each showed between 1 and 2 mgm. P per 100 cc., estimated at 1.5 to 1.7. Plasma P 7.3 mgm. per 100 cc. No urine.
7. Jan. 3, 1927. Female. Urine before experiment had 4 mgm. P per 100 cc. Fluids from two capsules each showed between 1 and 2 mgm. P per 100 cc., estimated at 1.7 to 1.8. Plasma P 6.0 mgm. per 100 cc.
8. Nov. 5, 1927. Female. 0.3 per cent paraldehyde and brain pithed. Capsular fluid between 1 and 2 mgm. P per 100 cc., nearer 2, estimated at 1.8. Plasma P 6.0 mgm. per 100 cc. Bladder urine accumulating during experiment had 8.3 mgm. P per 100 cc.
9. Nov. 19. Female. Brain pithed. Urine before experiment had about 1 mgm. P per 100 cc. Capsular fluid had 2 mgm. P per 100 cc. Plasma P 6.8 mgm. per 100 cc.
10. Nov. 21. Female. Brain pithed. Urine before experiment 1.8 mgm. P per 100 cc. Capsular fluid showed no P or less than 1 mgm. per 100 cc. Plasma P 5.7 mgm. per 100 cc.
11. Dec. 6. Female. Urine before experiment 3.2 mgm. P. per 100 cc. Capsular fluid much nearer 1 than 2, estimated at 1.3 mgm. P per 100 cc. Plasma P 5.4 mgm. per 100 cc.

May 29, 1926. Female. Urine before experiment had 8.2 mgm. P per 100 cc. Two capsular fluids had moderate protein clouds, about same as is given by Necturus' plasma diluted 1 to 50, showed about 3 mgm. P per 100 cc., deeper blue on precipitate. Third fluid had heavier cloud, color about same as 6 mgm. standard. Plasma P 6.2 mgm. per 100 cc.

Additional plasma analyses, not included in experiments of protocols, showed 7.0, 5.8, 5.8, 6.8 and 5.8 mgm. P. per 100 cc. plasma.

this amount. In one case the level was less than 1 mgm. P per 100 cc. with a plasma level of 5.7 mgm. per 100 cc. One experiment where protein was present is included. The higher phosphate content here is typi-

cal of protein-containing capsular fluids. Whether the deeper blue means that the phosphate has come through bound to the protein or that the same change in the membrane which rendered it permeable to protein also permitted the passage of non-protein phosphate complexes to which it was formerly impermeable, we do not know.

In most of our experiments not enough urine entered the bladder while the animal was on the board for an analysis to be made, even though the kidney circulation was excellent. This may be in part due to the short duration of the experiments. It must also be remembered that the ureters do not enter the bladder, but the cloaca, so that failure of urine to accumulate in the bladder is no indication of a failure of urine formation. In two cases, experiments 4 and 8, enough urine for analysis accumulated in the bladder while the animal was on the board; in one of these the urine P was between 3 and 4 times the glomerular fluid P, in the other case between 4 and 5 times.

Discussion. It is evident that in Necturus only one-fourth to one-third of the plasma phosphates as determined by conventional methods for inorganic phosphates can pass through the glomerular membrane. Eicholtz and Starling (1925) postulated that the inorganic phosphate of plasma in the dog exists as colloidal calcium phosphate to which the glomerular membrane is completely impermeable. As against their finding of complete impermeability to plasma phosphates we find a partial permeability, with normally circulating blood. This discrepancy may mean that the plasma phosphates in the dog are not at all able to pass the glomerular membrane normally, while a part of Necturus plasma phosphate can pass, or it may mean that the conditions of their experiments, as defibrination of the blood, may have altered the state of the phosphates or rendered the glomerular membrane abnormally impermeable. On the other hand the discrepancy may mean that no phosphate normally passes the glomerular membrane in either the dog or Necturus and that our finding of small amounts of phosphate is due to a slight injury to the glomerular membrane by the pipette, permitting the passage of phosphate while still not permitting the protein to pass. It is difficult to see, however, how the glomerular membrane could have been in any way injured in our experiments. In an active glomerular capsule of Necturus the fluid-filled space between the capillary tuft and the capsule wall is wide enough so that the pipette can easily be inserted and lie free in this space without at any time making contact with the capillary tuft.

As Grollman (1926) has pointed out, the results of experiments on ultrafiltration through collodion membranes, even when carefully controlled as to preparation of membranes, filtration pressure, etc., can be accepted as indicative of the ability of the various plasma constituents to pass living membranes only in so far as they do not conflict with data obtained by dialy-

sis through living membranes, since no one can say that the permeability of any given collodion membrane is identical with that of the glomerular membrane. Grollman finds that 50 to 80 per cent of the plasma phosphates in the frog pass through a collodion membrane. If this is also true of Necturus plasma it apparently means that the glomerular membrane is less permeable to certain of the forms in which phosphate exists than were the membranes he used.

The findings of these experiments do not answer decisively the question of a tubular secretion of phosphate. It is clear that if the glomerular filtrate were phosphate free at a time when the urine contained phosphate the question of tubular secretion of phosphate would be answered in the affirmative, but with even 1.5 mgm. P per 100 cc. of filtrate the presence of urinary phosphates, even at a considerably higher concentration than in the filtrate, could be explained by water reabsorption. Our single experiment, no. 10, in which no appreciable blue color appeared in the capsular fluid tube, is not decisive of this point, since no urine secreted during the experiment could be collected. An advocate of tubular secretion might find it easier to suppose that the tubules are adding phosphate to the glomerular filtrate than to suppose that, in an aquatic animal which has no conceivable need to conserve water, the tubules would actively reabsorb a proportion of the water equivalent to at least 75 per cent of the fluid filtered through the glomeruli, but it must be admitted that the matter is still one of speculation. We have a few data from perfusion experiments and from a comparison of concentration ratios of phosphate and urea which tend to favor the view of tubular secretion but the data are not yet extensive enough to justify a definite stand.

SUMMARY

The results of phosphate determinations on 15 capsular fluids from Necturus are reported. Fourteen of these showed from 1.3 to 2 mgm. P per 100 cc., one showed no P or less than 1 mgm. per 100 cc. The plasma P varied from 5.4 to 8.9 per 100 cc.; it is seen that only one-fourth to one-third of the plasma "inorganic phosphate" is diffusible through the glomerular membrane. The urine P varied from 1 to 8.3 mgm. per 100 cc.

BIBLIOGRAPHY

BENEDICT, S. R. AND R. C. THEIS. 1924. Journ. Biol. Chem., lxi, 63.
BRIGGS, A. P. 1922. Journ. Biol. Chem., liii, 13.
EICHOLTZ, F. AND E. H. STARLING. 1925. Proc. Roy. Soc., xcviii, 93.
GROLLMAN, A. 1926a. Journ. Gen. Physiol., ix, 813.
1926b. Journ. Biol. Chem., lxxii, 565.
WHITE, H. L. AND F. O. SCHMITT. 1926. This Journal, lxxvi, 220.

A STUDY ON THE MECHANISM OF THE STROPHANTHIN MYDRIASIS IN THE RAT

ROBERT DANE BARNARD

From the Department of Physiology of the University of Chicago

Received for publication January 13, 1928

Koppanyi and Sun (1) have reported a marked pupillary dilatation after instillation of strophanthin solutions into the eye of the white rat. They attribute the results to direct action of the drug upon the smooth musculature of the iris. Concentrated solutions of strophanthin (10 to 25 per cent) and repeated instillations for from twenty to thirty minutes were found necessary for maximal effect (2).

In light of the massive concentrations used by the previously mentioned investigators it was thought advisable to determine whether the mydriasis they reported was due to specific drug action or to other factors (i.e., the surface tension reducing property of the strophanthins) (3). The present investigation was therefore undertaken.

PROCEDURE. A series of substances exhibiting surface activity but differing among themselves in chemical structure was substituted for strophanthin in the procedure of Koppanyi and Sun (1).

Adult albino rats without pupillary inequality were used. The head was held between the thumb and forefinger of the left hand as in this way the palpebral fissure could be widened. Instillation was made into the right eye at three-minute intervals so that the conjunctival sac was kept brimming with solution.

RESULTS. All substances known to reduce surface tension with one exception (hexylresorcinol) gave rise to mydriasis upon continued instillation. The results with potassium oleate are discarded.

Both oral and intraperitoneal administration of large quantities of strophanthin (up to rapidly lethal doses) and the intraperitoneal injection of bile salts were devoid of pupillary action.

DISCUSSION. The constant occurrence of saponins with ordinary digitalis preparations will not permit any interpretation of the mydriasis following the use of this drug. In fact the active digitalis glucosides themselves reduce surface tension (4). It is therefore impossible by the use of digitalis to separate the effects of surface activity from those of specific drug activity.

The negative results with hexylresorcinol may be attributed to the olive oil vehicle employed for its instillation.

The results with resorcinol show that both hypertonic and hypotonic solutions are effective.

Failure to obtain mydriasis upon instillation of barium chloride solution is interesting since according to Sollmann (5) barium has chiefly those pharmacodynamic effects which characterize the digitalis group.

The inability of systemically administered strophanthin and of bile salts to produce pupillary dilitation may be explained on two bases: 1. That

TABLE 1

SOLUTION	NUMBER OF ANIMALS	TIME OBSERVED	MYDRIASIS	REMARKS
		minutes		
Digitalis	1	25	Medium	
Saponin 5 per cent	2	25–30	Medium	Hemolysis
Potassium oleate	1	30	?	Conjunctival opacity
NaGlycocholate, 4 per cent	3	15–20	Maximal	
Nataurocholate, 4 per cent	2	15–20	Maximal	
Hexylresorcinol (2 per cent in olive oil)	1		None	
Resorcinol, 10 per cent	2	5–8	Maximal	$\Delta = 1.69$
Resorcinol, 5 per cent	1	5	Maximal	$\Delta = 0.84$
Resorcinol, 2.5 per cent	1	15	Medium	$\Delta = 0.42$
KCl, 30 per cent	1	30	None	
$BaCl_2$, 10 per cent	2	30	None	
Glucose, 8 per cent	1	30	Very slight	
Glucose, 25 per cent	1	30	None	

TABLE 2

SOLUTION	PER CENT	DROPS PER CUBIC CENTIMETER $H_2O = 18$	SURFACE TENSION DYNES/CM.
Strophanthin	4	26	50.5 ± 1
Strophanthin	2	24	54.7 ± 1
Resorcinol	3.85	20	65.7 ± 3
Resorcinol	10	21.5	61.1 ± 3

should surface activity of the effective substances be the causative factor in the mydriasis, this surface activity is impaired by combination with substances having a tendency to nullify change in surface tension (e.g., cholesterol, etc.). 2. That the seat of action in this particular case is not readily reached through vascular channels.

It can be seen by the figures given for the surface tension of two strophanthin solutions (table 2) that in the case of the concentrations used by Koppanyi (2) the values would lie upon the horizontal asymptote of the

surface tension dilution curve. This fact would seem to eliminate surface tension as the cause of the mydriasis. However we do not know the surface area of the substrate and it is possible that this surface is sufficiently great as to require an initially high concentration of surface active substance to complete a final monomolecular layer. It may be that high concentrations are necessary to insure penetration of a small amount.

CONCLUSION

Since strophanthin lowers surface tension and many other substances exhibiting this physical property but differing in chemical structure (digitalis, saponin, sodium taurocholate, sodium glycocholate and resorcinol) produce mydriasis in the eye of the rat it is probable that strophanthin owes its mydriatic action at least to a great degree to its surface activity.

The author wishes to acknowledge his indebtedness to Drs. A. J. Carlson, T. Koppanyi and A. B. Hastings and to Mr. E. U. Still for valuable criticisms and advice.

BIBLIOGRAPHY

(1) Koppanyi and Sun. This Journal, 1926, lxviii, 358.
(2) Koppanyi. Personal communication.
(3) Clark. Journ. Pharm. Exper. Therap., 1914, v, 233.
(4) Trendelenberg. Arch. f. exper. Path. u. Pharm., 1909, lvi, 256.
(5) Sollmann. A manual of pharmacology. Philadelphia, 1926, 535.

THE EFFECT OF LOW PROTEIN AND PROTEIN-FREE DIETS AND STARVATION ON THE VOLUNTARY ACTIVITY OF THE ALBINO RAT[1]

F. A. HITCHCOCK

From the Laboratories of Physiology, Ohio State University

Received for publication December 2, 1927

In a recent paper (Hitchcock, 1926) it was shown that the activity of the albino rat is not appreciably affected by the addition of meat to an adequate diet. This would seem to constitute further evidence in support of the conclusions of Chittenden (1904) and others, that the ability to do work is not dependent upon the amount of protein in the diet, provided that an adequate amount of complete proteins is furnished. As a further investigation along the same line a group of experimental animals and their controls were selected according to the procedure already described (Hitchcock, 1926). The experimental animals were then put on a low protein diet consisting of whole wheat flour 92.5 per cent, cotton-seed oil 5 per cent, $CaCO_3$ 1.5 per cent, and NaCl 1 per cent. This diet was made up in cakes containing about 10 per cent water. We have computed that one gram of this food contains about 3.7 calories, 12 per cent of which is furnished by protein. The activity of the two groups for two months before and fourteen months after the beginning of the low protein feeding is shown in figure 1. Originally there were thirteen animals in the low protein group and eleven in the control group. All animals were adults from six to eight months old at the beginning of the experiment. In the low protein group there were four males and nine females while the control group was made up of nine females and two males. In the final data only animals which lived for at least six months of the experiment are considered. This eliminated four of the controls (one male and three females) and two of the low protein animals (both females). This leaves eleven animals in the low protein and seven in the control group. In the low protein group two animals died during the seventh month of the experiment, two during the eighth month, one during the ninth month, and one during the twelfth month. Five animals were left that lived throughout the entire experiment. In the control group two animals died during the twelfth month which leaves five that lived throughout the entire length of the experiment.

[1] This investigation has been aided by a grant from the Department of Nutrition of the Institute of American Meat Packers.

Considering the entire series, 61 per cent of the low protein animals died before the end of the experiment and 55 per cent of the controls, a difference which, considering the small number of animals in the series, is not significant.

Previous to the beginning of the low protein feeding the activity of the experimental group was well above that of the controls. However, coincident with the beginning of low protein feeding there appears to have been a more rapid decline in the activity of the experimental group than in the controls. By the end of the third month of low protein feeding the controls were showing greater activity than the experimental animals. From this point on to the end of the experiment the controls always showed the

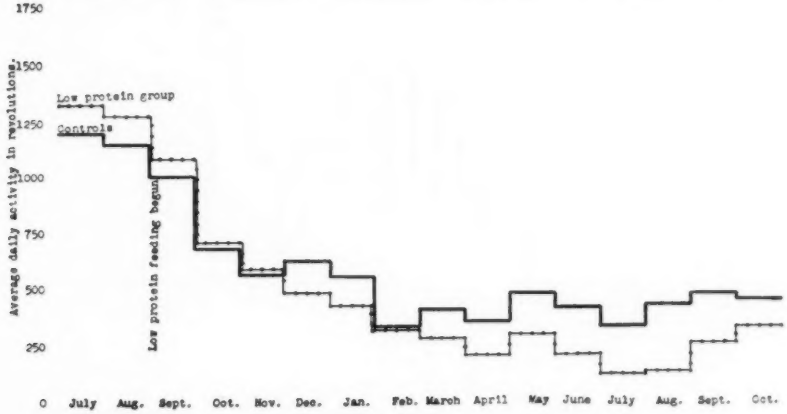


Fig. 1. Graph showing the voluntary activity averaged by months, of a group of eleven rats fed on a low protein diet and a group of seven control animals that were fed on the regular standard ration. Six low protein and two control animals died during the experiment so that there were five in each group at the end of the twelfth month.

greater activity. In only one month (the sixth of low protein feeding) did the experimental group even closely approach the activity of the controls. For the last eight months of the experiment, the activity of the low protein group was only 55 per cent as great as that of the controls. Since the two groups were kept under conditions identical in all respects except diet, and since the depression of the activity of the experimental group was coincident with the beginning of low protein feeding, the conclusion that the low protein diet depressed voluntary activity seems justified. Although theoretically this low protein diet is adequate and contains an amount of protein well above the minimum requirement of the white rat, our experience has shown that it is by no means optimal. Young rats show a very

slow rate of growth on this diet, and mother rats seldom succeed in rearing young on it. These facts lend support to the theory that the decreased activity on the part of the low protein animals was an indication of a general lowering of the well-being of the animals resulting from a ration, which, while not actually deficient in any way, was still far from optimal.

There is a marked difference between the effect just described and that produced by the total absence of protein from the food. Fifteen animals, all adult males, that had been in the revolving wheels on our standard ration for at least three months, were put on a protein-free food consisting of glucose, corn starch, cotton-seed oil, and appropriate amounts of $CaCO_3$ and NaCl. The effect on their activity is shown in figure 2. It is quite

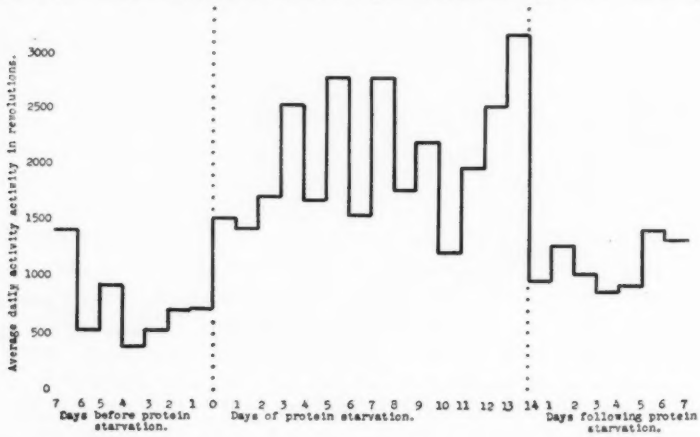


Fig. 2. Graph showing the average daily activity of a group of fifteen adult male rats for seven days preceding, during and seven days following a period of fourteen days protein starvation.

evident that protein starvation acts as a strong stimulus to activity. The average daily number of revolutions run by these animals for the seven days preceding the period of protein starvation was 744 (the wheels are 18 inches in diameter). During the fourteen days that the animals were on protein-free food the average daily number of revolutions run was 2062. This is an increase in activity of 177 per cent. For the first seven days following the close of the two weeks of protein starvation, the average number of revolutions run daily was 1121, which was a decrease over the preceding period of about 46 per cent. The conclusion, therefore, seems justified that the complete absence of protein from the food acts on the white rat as a powerful stimulus to activity.

Complete fasting also has a marked effect in increasing the activity of

the white rat. It has already been reported by Hoskins (1925) that starvation increased the voluntary activity of castrated rats as much as 200 per cent. It would be expected, therefore, that a similar effect would be present in normal animals. Twenty-five adult rats (14 males and 11 females), all of which had been kept constantly in revolving cages for more than three months, and fed on our regular stock ration, were subjected to a three-day complete fast, at the end of which they were again placed on the stock ration. The average daily activity of the group for five days preceding, during, and five days following the fast is shown graphically in figure 3. The average daily activity for the five days preceding the fast was 2331 revolutions, while during the fast the activity of the group in-

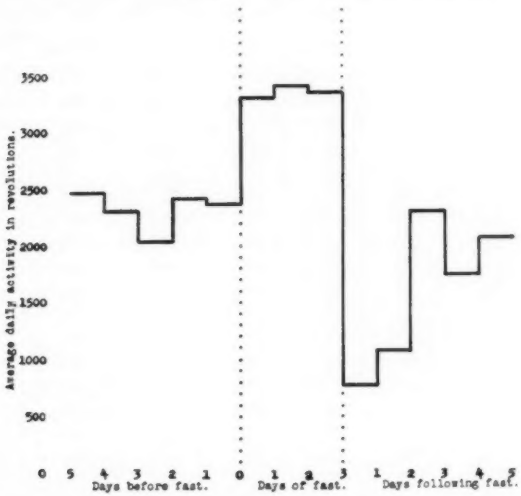


Fig. 3. Graph showing the average daily activity of twenty-five adult rats for five days preceding, during, and five days following a three day complete fast.

creased 44 per cent or to an average daily level of 3363 revolutions. Immediately following the end of the fast there was a very marked depression of activity. However, by the third day it was back to a figure comparable to that which preceded the fast. The average for the five days immediately following the fast was 1594 revolutions daily, more than 52 per cent less than the activity during the fast. This is a much smaller increase in activity than that reported by Hoskins (1925) for starved castrated rats. The castrated rats in Hoskins' experiment, however, started from a general level of activity considerably lower than that of the animals used in this experiment. It is also possible that in the absence of the sex stimulus the hunger stimulus may be stronger than in a normal animal.

Richter (1927) has recently published a review of the work on animal activity in which he stresses the periodic nature of voluntary activity and attempts to correlate it with periodically functioning organs. He has definitely established a relationship between spontaneous activity and

TABLE 1

Data from which has been calculated the caloric value of the weight lost by albino rats during a fast

RAT NUMBER	WEIGHT AT BEGINNING OF FAST	WEIGHT AT END OF FAST	GRAMS LOST PER DAY	SQ. CM. OF BODY SURFACE	GRAMS LOST PER SQ. M. OF BODY SURFACE	AVERAGE DAILY ACTIVITY IN METERS	COMPUTED DAILY CALORIC REQUIREMENT IN CALORIES PER SQ. M. OF BODY SURFACE	CALORIC VALUE OF 1 GRAM OF WEIGHT LOST
1	308	267	13.7	495	276	1,120	1,149	4.16
2	200	167	11.0	367	300	11,200	1,493	4.98
3	184	163	7.0	356	197	6,650	1,391	7.06
4	217	186	10.3	391	264	23	936	3.55
5	198	156	14.0	358	391	9	914	2.34
6	177	138	13.0	332	392	2,352	1,235	3.15
7	224	181	14.3	392	366	11,961	1,508	4.12
12	204	169	11.7	371	314	1,386	1,173	3.74
13	280	222	19.3	452	428	11,093	1,492	3.49
14	221	191	10.0	396	252	14,793	1,898	7.53
15	210	180	10.0	381	262	17,985	2,085	7.96
16	311	281	9.7	505	192	355	1,058	5.51
17	335	302	11.0	528	208	896	1,131	5.44
18	356	309	15.7	545	288	8,097	1,428	4.96
19	223	186	12.3	394	313	1,022	1,143	3.65
20	348	312	12.0	543	221	3,528	1,290	5.84
21	330	281	16.3	516	316	8,246	1,432	4.53
22	230	194	12.0	404	297	8,283	1,433	4.83
23	281	256	8.3	472	176	2,063	1,219	6.93
24	375	340	11.7	571	204	966	1,138	5.58
25	390	355	11.7	588	199	1,727	1,198	6.02
26	369	325	14.7	551	267	490	1,080	4.04
27	245	223	7.3	431	169	1,185	1,157	6.85
28	307	272	11.7	497	235	93	985	4.18
29	339	297	14.0	528	265	2,193	1,226	4.62
Average..	274.5	238.1	12.1	454.6	271.7	4,708.6	1,287.7	5.00

hunger contractions of the stomach. In the light of this work, it would seem probable that increased activity of the rats during a complete fast could be accounted for by assuming that during the fast, hunger contractions occurred with greater frequency and also no doubt, with greater intensity than normally. By similar reasoning we reach the conclusion

that the hunger contractions in rats on a protein-free diet are increased either in intensity or in frequency or in both, in spite of the fact that the total bulk of the food consumed by the animals during this period was fully as great as that eaten while they were being fed an adequate ration. So far as I have been able to ascertain, no work has been done on the effect of inadequate diets on hunger contractions. The results reported here would seem to indicate that such investigations would be well worth undertaking.

It is possible by the use of the formula $Y = 855 + 28.58\sqrt[3]{X}$, (in which Y is calories per square meter of body surface and X is activity in meters) which has been suggested as a means of computing the total caloric requirement of the albino rat (Hitchcock, 1927), to arrive at a figure which approximately represents the caloric content of the weight lost by these rats during the three-day fast. In table 1 the computed value of Y for each rat included in this experiment is shown, together with the data necessary in making the computations. The number of grams lost per square meter of body surface is also shown for each rat. By dividing this figure into the computed daily caloric requirement expressed in calories per square meter of body surface, the approximate caloric value of each gram of body weight lost has been calculated. This figure is shown in the last column in table 1. There is a wide degree of variation in these figures, for they range all the way from 2.34 calories per gram for rat 5 to 7.92 calories per gram for rat 15. The average is 5 calories per gram of body weight lost, while eight animals, or about one-third of the entire group, fall between 4.5 and 5.5 calories per gram, and thirteen, or more than half of the entire group, fall between the limits of 4.0 and 6.0 calories per gram of weight lost.

These figures probably do not represent the true caloric value of the weight lost for the individuals in the series, for to assume that they did would be to assume a caloric requirement for each individual exactly similar to that determined as the average for a large series in which it was pointed out that the range of variability was very great (Hitchcock, 1927). This method of calculation, therefore, incorporates in the one figure not only the variation in the caloric value of the body weight lost, but also the variation in the individual caloric requirement. However, it seems probable that the average caloric requirement of this group of animals would be the same as the average of the group on which the mathematical expression used in these computations was based. The conclusion seems justified, therefore, that five calories per gram is approximately the average caloric value of the weight lost by the rats used in this experiment during a three-day fast. Assuming that all of the energy used was furnished by fat, an average of about 6.7 grams of fat must have been consumed in order to furnish the 60.5 calories which is the average daily energy consumption

of the rats in this group. That would mean that about 45 per cent of the weight lost by these animals during the three-day fast period was water. It seems more probable, however, that at least part of the energy used was furnished by stored carbohydrate. If we assume that equal quantities of fat and carbohydrate were used, then about 4.7 grams of each would have been needed to furnish the energy used. This would leave 2.7 grams of water lost which is about 22 per cent of the total weight lost. It seems probable, therefore, that the material used to furnish energy during the fast contained a much lower percentage of water than ordinary body tissues.

SUMMARY

1. A diet containing about 12 per cent protein, all of vegetable origin, when fed over a long period depresses the activity of the albino rat.

2. Both protein starvation and complete starvation for short periods act as powerful stimuli and increase activity to a marked extent.

3. It has been computed that the average caloric value of weight lost by albino rats during a short fast is approximately 5 calories per gram.

BIBLIOGRAPHY

CHITTENDEN, R. H. 1904. Physiological economy in nutrition. F. A. Stokes & Co., New York.

HITCHCOCK, F. A. 1926. This Journal, lxxix, 206.

1927. This Journal, lxxxiii, 28.

HOSKINS, R. G. 1925. Endocrinol., ix, 403.

RICHTER, C. P. 1927. Quart. Review Biol., ii, 307.

THE RELATION BETWEEN THE THRESHOLD OF NERVE RESPONSE AND POLARIZATION BY GALVANIC CURRENT STIMULI

GEORGE H. BISHOP

From the Department of Physiology, Washington University School of Medicine, Saint Louis

Received for publication December 27, 1927

In the following paper a correlation is attempted between the stimulation of nerve by galvanic currents and the physico-chemical properties of nerve, in particular its polarizability as an electrolytic conductor. Galvanic stimuli are suitable for such experimentation, on account of the relatively simple relationships existing between the amplitude and duration of a constant applied potential on the one hand, and on the other, threshold and polarizability characteristics of the nerve. Present conceptions of this relationship are due particularly to the work on chronaxie by Lapicque and his co-workers (1926). Nerve tends to respond at threshold to a constant quantity of current, and this is reconcilable with the conception that it responds when some critical interface has become polarized to a specific counter EMF by any applied current stimulus; for in general the counter EMF due to current flow across a polarizable structure is a function at any time of the quantity of current that has flowed. The question, how polarization as such is involved in the *response* of a nerve, is beyond the scope of this paper.

It is generally inferred that in the excitation of nerve, the permeability of the nerve membranes to the electric current, that is, to ions, increases. If the permeability of the nerve membrane is altered, i.e., if the membrane polarizability changes, during stimulation, before the time of response, the counter EMF should be either discharged or should increase at a different rate. This should be registered in the form of the polarization curve. Such a change has not so far been satisfactorily established.[1]

[1] A change in permeability of nerve due to ordinary stimulation does not appear to have been satisfactorily demonstrated. Samoijloff (1925) following Verzar (1912) reports a change in polarization as a result of stimulation in strongly polarized nerve, a change which becomes evident as a failure of the potential to return to a previous level for some time after response. This change is evident *only after* response, not during stimulation. That the change is cumulative in Samoijloff's records, the effect from one stimulus outlasting several successive responses (see his fig. 3), differentiates it from the typical irritability phenomena of nerve, which are consummated more promptly.

The building up of the characteristic counter EMF of polarization is to be assigned to the concentration of charged ions at an interface, and the increase of this counter EMF, for simple cases, under a constant applied potential, takes place in accordance with a simple exponential law, that which also applies to the charging of a condenser. If we plot potential on the y axis, against time on the x axis, the curve of polarization is logarithmic. The form of this curve is not essentially different if some ions flow through the membrane, being still logarithmic in character, though its time relations may be changed, and its maximum reduced.

In the logarithmic formula for the polarization of any given simple structure, ($y = (1-e^{-x})$ in simple form) the quantity of current that has flowed at a given time x is directly proportional to the counter EMF y. Lapicque, building on the foundation laid by Nernst (1904), Hoorweg (1892), Weiss (1901), etc. found that such an exponential formula fitted nerve phenomena approximately, in simple conditions (Lapicque, 1907). Whether the polarization counter EMF *per se* is the important factor, or whether the amount of electrolysis produced by the current, is not determined. More recently Lapicque (loc. cit. 1926 and in papers there cited) has questioned the adequacy of polarization as a complete measure of the nerve stimulus. Nerve does not follow such a law exactly, but nerve is not so simple a structure as the exponential formula is strictly applicable to, and deviations from the rule may possibly be due to the physical complexity of the nerve structure. The polarization hypothesis seems a potentially exact and simple theoretical explanation, provided we can account for the deviations which occur in the investigation of any given nerve. Before modifying or complicating this potentially simple hypothesis to bring it into more strict concordance with observed data, it seems advisable to study the physical properties of the nerve as a reacting conductor, and the effects of its reactance upon electrical measurements of its irritability.

1. *Graphic representation of polarization as measure of nerve stimulus.* The theoretical expectation for the "threshold" polarization of a simple structure under a constant potential difference and through a constant resistance, may be put graphically into the form of figure 1. The family of polarization curves $a' - e'$ are plotted from the formula $v = N\ (1-e^{-t})$, the applied voltage v being along the Y axis. They differ from each other only in the factor N, which is expressed along the Y axis as a multiple of the rheobasic voltage taken as unity. Threshold is assumed, for convenience, at a counter EMF (line LM) equal to 95 per cent of the asymptotic value attained under the lowest or rheobasic voltage.

Since the maximum polarization EMF resulting from any applied voltage (asymptote value) is proportional to that voltage, the asymptote of any polarization curve may stand also for the applied voltage producing

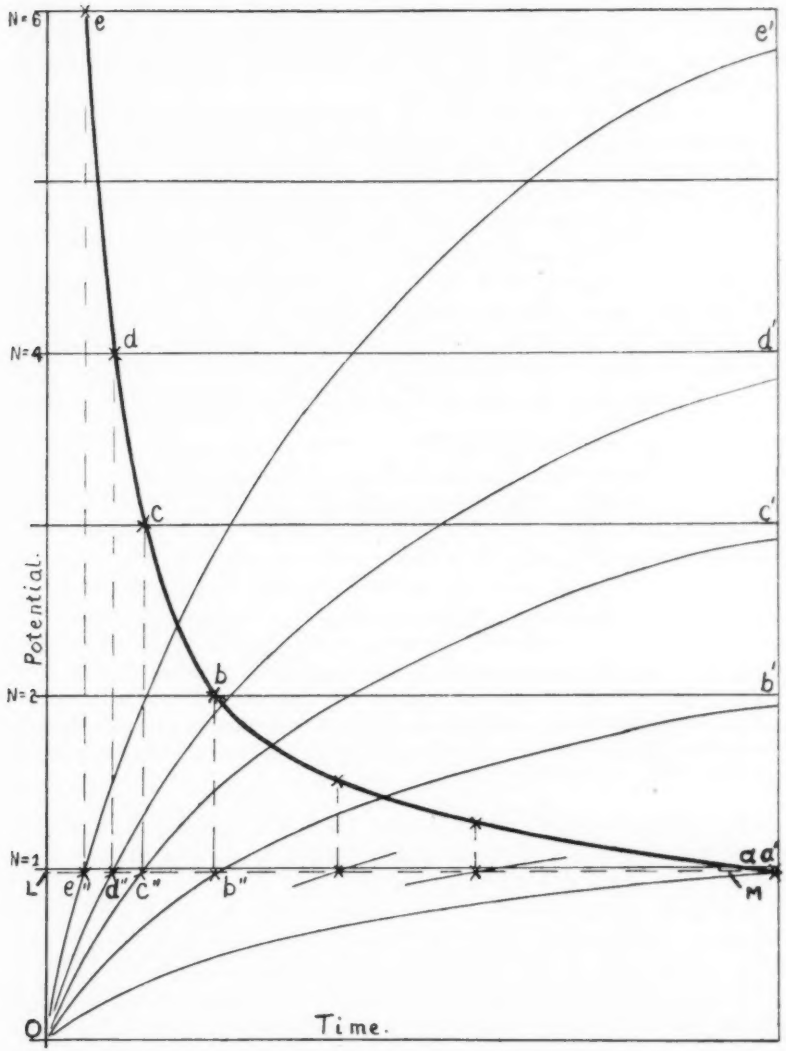


Fig. 1. Graphic representation of "threshold" polarization under different potentials. Analysis in text.

that curve, without invalidating a general statement of the case. This is equivalent to assuming that the counter EMF finally equals the applied, no current leaking through the membranes; that is, the nerve becomes a condenser, charged through resistance.

A point along any plotted curve at which such curve of counter EMF crosses the threshold line L-M, locates the time at which threshold counter EMF is attained under the corresponding applied potential. A plot of the applied potentials (values of N) against these corresponding times (a'', b'', c'', etc.) gives the theoretical chronaxie curve through the points a, b, c, etc. In this figure, the area between a given polarization curve such as $o\ c''$, its asymptote ($N=3$), the y axis and the line $c\ c''$ represents the potential times duration which is proportional to the quantity of current that has flowed, the effective potential being the applied minus the counter EMF. This quantity is proportional to the distance from c'' to the x axis, and this distance is equal for each curve involved. Thus

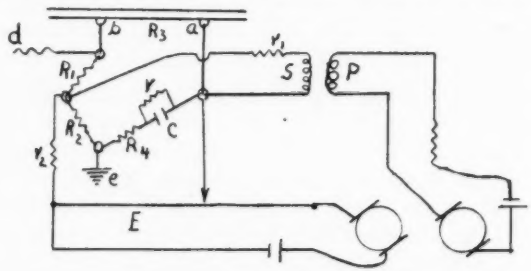


Fig. 2. Diagram of nerve polarization balanced by capacity and resistance in Wheatstone bridge, to obviate distortion of the record by stimulating potentials. Analysis in text, and see figures 3 and 4.

the quantities of current that have flowed through the polarizable structure at threshold times for different applied voltages are equal, and the corresponding counter potentials attained are equal.

This figure is substantially a plot of Lapicque's formula for the theoretical chronaxie curve of nerve (1907) or of Hill's equation (1910), reduced to the same terms by the omission of more or less arbitrary constants.

Put in this perhaps oversimplified form, it would be an exception to the general rule of biological complexity if the polarization of nerve by a galvanic current followed this rule exactly; yet a lack of exact fitting of the formula to the experimental curve has prompted physiologists to search for stimulating effects of current flow other than polarization.

The physical circuit through which current flows when the average nerve is stimulated is sufficiently complicated to cause the time-strength relationships to vary from those predictable by any formal theory so far

developed (with the possible exception of Hill's). In a previous paper (Bishop, 1927, fig. 2) the physical structures in a nerve through which current must flow to stimulate any given nerve axon have been diagrammed in terms of capacity and resistance. This stimulus is obviously not the total current which flows through the stimulating electrode. However, it has usually been tacitly assumed that it is a proportional fraction of it; but even this may be erroneous, and such a difference as it is reasonable to assume will account for wide deviations from a simple theoretical behavior.

For instance, it is conceivable that when a constant potential difference is set up between two points on a nerve surface, one polarizable element (e.g., an axon of a given size) in the nerve might polarize with a counter EMF that followed a simple theoretical curve, with constants characteristic of this element, while another component of the current would meet with resistance, polarizable or otherwise, whose constants were different. The sum of these two resistance effects might not follow the simple law for a single polarizable structure, even though each by itself would follow it strictly. If the same current traversed the two structures strictly in series, the curve would still be expected to be simple, but since they are in part effectively in parallel, it should not. This is equivalent to saying that in a system of parallel conductors, poorly insulated, the reactance of each may affect the current flow through the others, in accordance with some complication of Kirchoff's laws for a divided circuit.

2. *Change in irritability of nerve during application of a constant potential difference.* We cannot measure the current through a given axon directly, but we can obtain a measure of its effect at successive times. Its effect, before the nerve's response, is to make the nerve easier to excite by a superposed stimulus. It is of interest therefore to follow the "physiological" effect of a constant applied potential on the irritability of the nerve axon in the period preceding the response, ignoring for the time being the total current that will flow through the whole nerve, which may not be an exact measure of that which flows through the given axon. By means of the cathode ray oscillograph and amplifier, we may obtain evidence (Bishop, Erlanger and Gasser, 1926) as to the form of the change in irritability in nerve due to passage of a current, separate from the response that customarily follows it. This evidence is derived as follows. Through a green frog sciatic nerve a just subthreshold galvanic current of 0.003 second or more duration from a constant potential source is passed by way of calomel electrodes at intervals of about $\frac{1}{10}$ second, synchronized with the time line of the oscillograph. The circuit may be so arranged that the polarizing effect of the current appears on the oscillograph apparently in a constant position where it can be photographed or observed at leisure. The nerve is placed in a Wheatstone bridge and balanced for polarization and resistance so that the polarizing potential is recorded

merely by a short shock-like deflection or "escape" at beginning and end, the main duration of potential being balanced out to the smooth zero or base line of the oscillograph (fig. 2). The phenomena can then be observed as they appear under the stimulating cathode, by leading from the bridge circuit into the amplifier input. The bridge technique has been described elsewhere (Bishop, 1927).

A break induction shock is sent over the electrodes which carry the galvanic current and by a slide wire in series with the primary coil, regulated to give a just threshold reaction when the galvanic current does not flow (fig. 2). With the galvanic current flowing, the strength of this shock can be reduced by reducing the primary current to a point where the now summated subminimal stimuli of shock and constant potential result in a just threshold stimulus following the shock. The shock is then placed at different times during the subliminal flow of current from the constant potential, and these times and the strength required for summation to threshold recorded. Time can be measured to 0.000,02 second, and the strength of shock is taken to be proportional to the amperage flowing through the primary before the break, determined by calibration of the wire in series with the primary with the interrupter running. The effective shock duration (time from beginning of shock to start of action wave) is taken as 0.000,06 second, which has been found repeatedly to be a reasonable value for our stimulating coil at threshold strength for frog nerve. The time of threshold stimulation with the rheobasic constant potential is for frog nerve 2 to 3σ at 25°C., compared to which the duration of the shock is very brief.

It is observed that even immediately before the threshold reaction to the rheobasic potential above, the shock has to have appreciable strength to increase this reaction. This we assume to be due to the fact that the potential of the shock is in parallel with that of the constant potential source, and thus does not add to the effect of the latter unless its potential rises above the constant potential. Consequently there is subtracted from all shock strengths the strength of shock which is just ineffective at the time of reaction to the constant potential. This may be shown to introduce no appreciable error. The amount of current flow above a certain fixed potential level, that of the constant applied potential, from different shock potentials, should be proportional to the shock potential above that level, quite as in a condenser, where the quantity of current discharged is proportional to the change of potential of the condenser. The correction is justifiable in so far as the shock discharge parallels a condenser discharge, which, for a very sudden break of the primary and high secondary resistance, is very close.[2]

[2] A more strict analysis of the mutual interaction of constant potential and shock may be made as follows. The constant potential is applied to the nerve, and to the

The effective strengths of shock plotted against time of current flow from the constant potential, are diagrammed in figure 3. The threshold strengths of shock and constant potential are arbitrarily converted to the same value for convenience in plotting, and are laid off below the x axis as denoting negative potentials. The strength of shock necessary to obtain threshold summation (distance from line $a\ d$ to curve $o\ d'$) decreases with duration of galvanic current, but not in linear fashion. To the amount of stimulation the constant potential has accomplished in the axons of lowest threshold at a given time, must be added an amount meas-

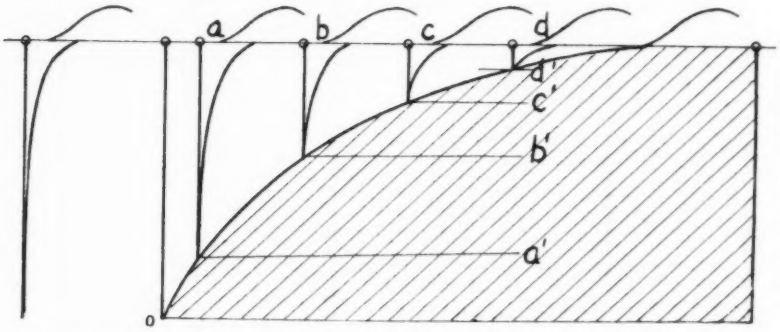


Fig. 3. Diagrammatic plot of summation of constant potential and induction shock potentials to threshold value, at successive intervals before response to constant potential alone. Analysis in text, and see figure 4.

ured by the shock value for that time, to give a constant threshold value. Since the effective shock time is very short, we may without serious error

coil in parallel, through electrodes to which the coil also leads. The secondary coil builds up, due to the current through it, a specific degree of self inductance. When the primary break occurs, the induced current passes from the coil through the nerve in the same direction as from the constant potential, but through the coil itself in the opposite direction. The inductance due to flow of current through the coil from the constant potential thus opposes the discharge of the coil, by a constant potential value, however the value of the induced coil potential may vary. This constant, represented by the induced potential necessary to stop the flow of current through the coil from the constant potential source, should be subtracted from all shock strengths, since it is not effective in sending current through the nerve. An analogous argument applies to the shunting of the nerve by the galvanic current circuit.

This argument ignores two factors, first, the changing counter EMF of the nerve, which however is very slight compared with the shock potentials in question, and second, the resistance in the stimulating circuits, r_1 and r_2, which complicate the result in so far as they are large compared to the total nerve resistance. The latter factor merely requires that a smaller but still constant shock value would be subtractable, which would still be measured by the shock strength at rheobasic threshold.

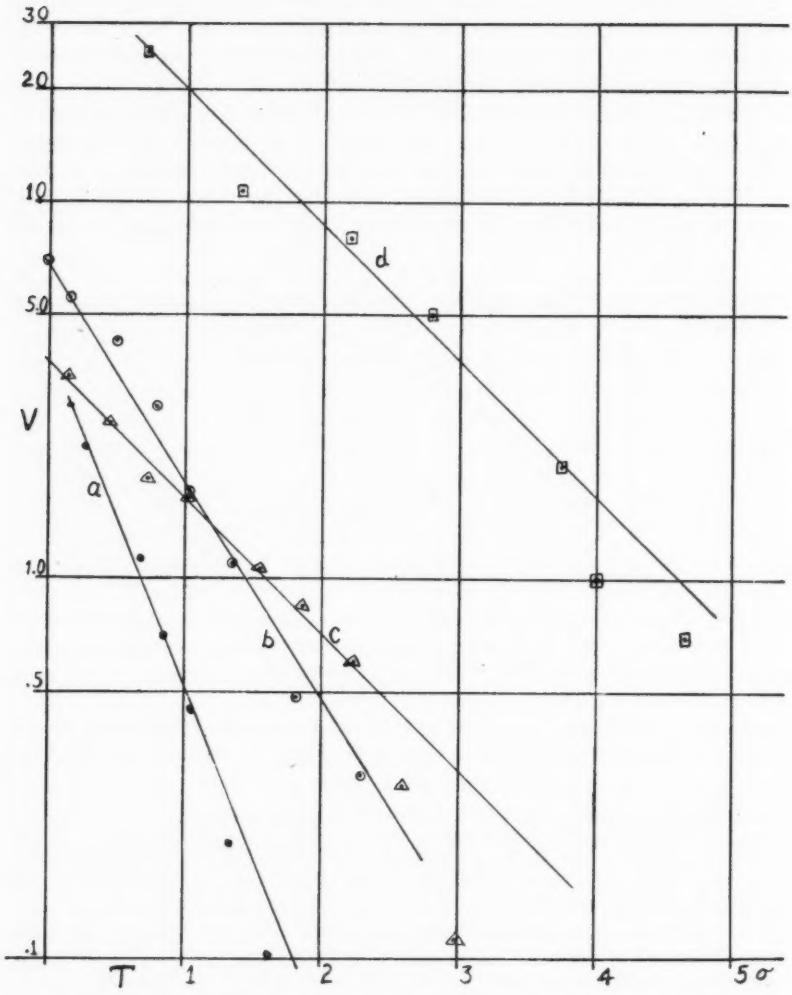


Fig. 4. Plot of logarithm of shock strength at threshold (y axis) against time during galvanic current flow. Curves a, b, c, green frog sciatic nerves, threshold to most irritable fibers of first or α wave. Curve d, threshold to most irritable fibers of second or β wave, observed after separation out from first wave with conduction. Shock strengths arbitrary values, measured by amperage in primary coil at break, and are not comparable from one nerve to another. Further details in text.

assume it to be instantaneous, and the stimulus to be delivered either at the end or at the beginning of the effective shock duration. The difference amounts only to a horizontal displacement of the whole curve, with no essential difference in form.

Now if we plot the points obtained by experiment on semilogarithmic coördinates they fall symmetrically about a straight line, except for the later points on the curve, for which the shock values appear to be too low

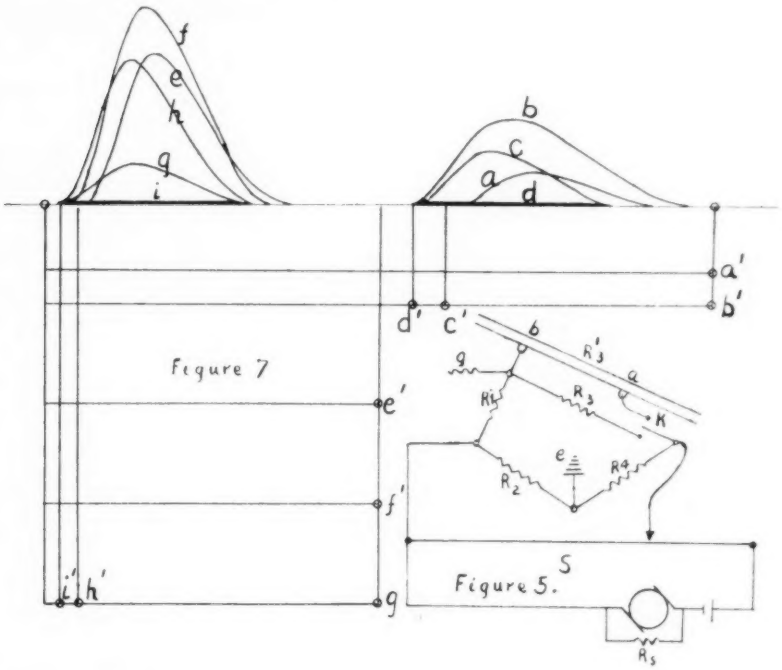


Fig. 5. Wheatstone bridge diagram for records of figure 6.

Fig. 7. Diagram for analysis of change in form of recorded action potential with change of form of galvanic current stimulus. See text. Applied potential below base line, form of recorded action potential above.

to fit an exponential curve (fig. 4). This deviation corresponds to the fact that a rheobasic current stimulates, not after an infinite duration (asymptote value of a true exponential curve), but considerably previous to this. Further, the change in the nerve starts along a curve corresponding to polarization of greater than threshold value, and appears to deviate from this curve chiefly in its later portion.

3. *Direct record of the polarization curve of nerve.* The curve of change of

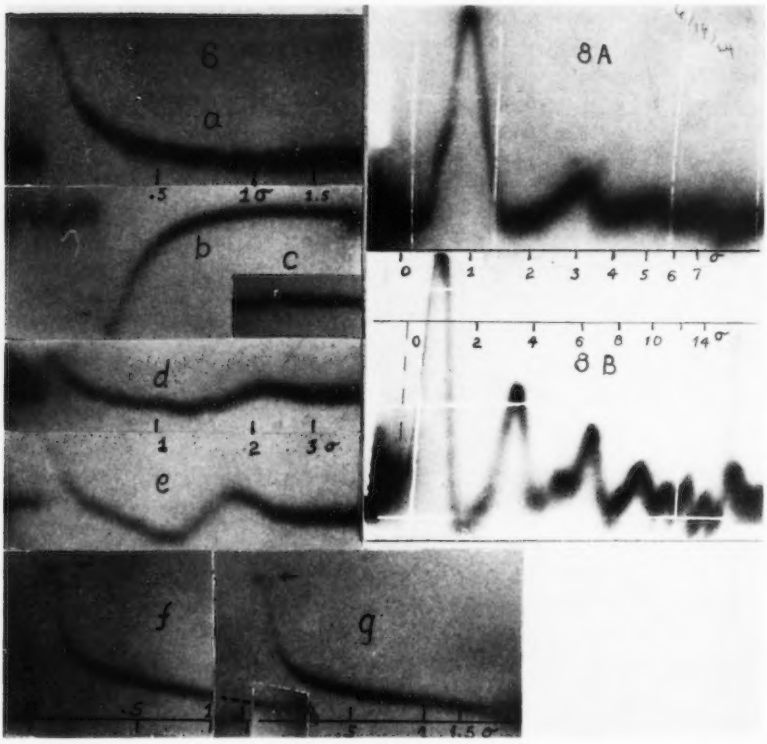


Fig. 6. *a*, Polarization curve green frog sciatic nerve, below threshold, make of current, semilogarithmic coördinates, time in 0.000,5 second. *b*, break of current. *c*, break of current with nerve replaced in bridge by non-capacitative resistance, as check on bridge reactance. *d*, response, slightly above threshold, superposed upon polarization curve, shunted to reduce ripples due to commutator wheel contact carrying strong current. *e*, same as *d*, stronger current. *f*, polarization curve of green frog nerve, break of current after long duration of flow. *g*, same, short duration of current before break. The difference between *f* and *g* amounts to a shift of base line (asymptote), the two curves having otherwise the same form. About two-thirds natural size.

Fig. 8. A. Green frog sciatic nerve, repetitive response to a constant potential, medium stimulus. Nerve balanced in Wheatstone bridge leaving slight distortion upon rising phase of first response, and following the fourth. Time in 0.001 second (5,000 ω). About two-thirds natural size.

B. Same as above, stronger stimulus. Time in 0.001 second (10,000 ω).

irritability thus has somewhat the form of a counter EMF polarization curve in general. The polarization curve of the whole nerve may be recorded directly as follows. It has been stated above that the nerve acts like a leaky condenser, or one shunted by resistance. The polarizability of nerve can be approximately balanced out in a Wheatstone bridge by capacity with parallel resistance, the whole in series with a second resistance. Now to reach a given axon, current must first pass through whatever tissue surrounds it, such as connective tissue, or other axons. This corresponds to the R_4 of figure 2. The r corresponds either to the leak of current through the nerve membranes, or to flow of current along the axon through other paths after traversing R. R includes also the internal resistance of the axon. If we ignore for the present the assumption that polarization takes place elsewhere than at the axon surface, c (at the connective tissue, for instance) which would only be analogous to charging condensers in series, the curve of polarization of this circuit should still be approximately logarithmic.

The nerve placed in a Wheatstone bridge is balanced for a steady current by non-reactive resistances (fig. 5) By a two-way key, K, a variable resistance, R_3, can replace the nerve R'_3, as a non-capacitative control (fig. 6*b* and *c*). A shunt R_s relatively small compared to the bridge resistance but large compared to the slide wire resistance reduces a distortion due to capacity effects of the potentiometer leads to a negligible value. The difference between 6*b* and *c* is a record of the transient potential imbalance during the process of nerve polarization, that is, it plots the curve of polarization of the nerve. In the record 6*b* shown, the break of the current instead of the make was dealt with, because during the flow of current, variable contact on the commutator wheel causes ripples in the line, as shown in 6*a*, which are not balanced out in the bridge because they are transients to which the polarizable nerve reacts. They do not appear in the record from the non-reacting R_3.

Upon break of the current, the counter EMF of polarization shows as an immediate change of potential, the now imbalanced counter EMF of the nerve, whose true maximum is probably masked by the reactance of our amplifier. The record of the rise of an "instantaneous" imput potential reaches 95 per cent of the maximum in 0.000,03 second, at which interval after the break the polarization curve has already started downward.

4. *Relation of nerve threshold to its polarization curve.* We may now increase the potential to a threshold value (fig. 6*d*). The action potential is recorded because it causes a potential imbalance in the bridge. The action potential, at the make of a threshold strength current, may be seen to arise only after the curve of polarization by the stimulating current approaches very closely to its maximum, as indicated by the return to zero

of the initial potential imbalance in the bridge record. A visible threshold response takes place slightly before the maximum is reached.

The curve of irritability change (fig. 3) and of polarization (fig. 6*d*) reach their maxima (at least their effective maxima) at approximately the same time, that is, at the beginning of the response to a threshold or rheobasic current. Since both are approximately logarithmic, they are *approximately* the same curves, with exceptions to be noted below (part 5).

As the applied potential is further increased, the action potential wave becomes higher, and moves forward upon the polarization curve (fig. 6*e*). It can now be seen to be distinctly diphasic, as should be expected, since the nerve was killed at neither electrode. That the second phase is opposite in direction to the first furthermore indicates more than a permeability change in the nerve, whether this takes place or not. If a permeability increase occurred upon excitation, the potential should tend to change twice in the same direction as the excitation passed from beneath the stimulating cathode, to beneath the anode, since there would occur two phases of decreased resistance in the same arm of the bridge. The reversal of the potential here indicates an active *potential* change under each electrode, and whatever passive resistance change may occur cannot be differentiated in the record.

5. *Relation of duration of galvanic current to form of polarization curve.* Polarization curves thus directly recorded from frog nerves with connective tissue sheath intact, are not simply exponential. It may be inferred that the connective tissue offers a complication of the circuit. Recorded from long bullfrog spinal roots, the approximation is close. On comparing curves, however, after varying durations of the galvanic current (fig. 6*f* and *g*), a further complication appears. After a very short duration of current flow, *g*, the curve approximates closely to the theoretical. By short duration is meant, just sufficient time to allow the make of the current to arrive at an approximately maximum polarization, which is accomplished in about 2 sigma at 25°C. With longer durations the curve deviates more and more from the theoretical, by developing a long "tail," i.e., a slower return of the latter portion of the polarization potential toward zero than would be expected from the rate of the earlier portion. Such an event should be expected if the longer durations had altered the nerve (possibly by electrolysis) relatively permanently, so that the immediate polarization discharge took place toward a new base line, this base line sloping gradually to the initial zero. This effect is not so marked with quantities of current that just fail to stimulate, unless of prolonged duration. That is, just subthreshold potentials give a relatively simple polarization picture, and there is no reason to suppose that slightly superthreshold potentials would do otherwise; the action potential, however, overlaps the polarization record.

6. *Changes in form of the recorded potential with increase of galvanic current stimulus.* Experiments reported briefly heretofore (Bishop, Erlanger and Gasser, 1926) on responses to galvanic stimuli, may be analyzed here in the light of the foregoing results. If a galvanic current of long duration and threshold strength is passed at intervals across the bridge, the nerve will respond each time with a small potential wave, only the most irritable fibers being stimulated (fig. 7*a*). If a larger number of fibers are stimulated by a slightly greater current, *b*, the resultant wave even at the locus of stimulation, is of longer duration than when fewer fibers are stimulated, or when all are stimulated by an induction shock. Its start is also earlier than in *a*. The different fibers are being stimulated at different times. The duration of the current flow is now shortened, *c*. As the end of the stimulating period falls progressively earlier, when at a point somewhat after the start of the wave, the wave decreases in height and shortens from behind forward, the start remaining stationary. Fibers of relatively high threshold have now failed to be stimulated, while those stimulated are the fibers previously stimulated at the lowest current strength, and at a later time. Exactly as the end of the stimulus passes through the start of the wave, *d*, the latter disappears altogether, indicating that the fibers of lowest threshold, and presumably all others, respond with no measurable delay, at the instant when adequate or threshold stimulation has been accomplished. This disposes finally of the possibility that *decrease* of current enters into the stimulation, for at threshold current for the most irritable fibers, the break of the current does not stimulate the next most irritable fibers, when the least increase of the current will.

As the current is increased in strength, *e*, the wave increases in amplitude and moves earlier, and finally becomes shorter, like that from an induction shock, when all fibers are stimulated in approximately the same phase. If the current is made stronger yet, repetitive responses may appear. Still further increase now causes the action potential wave to become lower, until it cannot be distinguished in the record, due to the cathodal depressing effect of the polarizing current. At this stage the potential wave is present at full amplitude, at other points in the nerve. It presumably propagates from a region away from the cathode to which current of stimulating value has spread. Now as the duration of stimulating current is shortened back toward the time when the action wave previously started, *h*, the wave reappears, increasing to its former maximum. With still further shortening, the wave falls lower, and fails completely, *i*, as the end of the stimulus passes the start of the wave. Strengthening the stimulus may again stimulate at this duration, etc.

It is apparent that at the cathode the current is exerting two distinct effects on the nerve, first, to stimulate, second, to depress in amplitude,

and that with increasing strength, and sufficient duration, the latter overtakes the former, until the nerve is depressed as it responds. The first cathodal effect is to stimulate, as the nerve polarizes, even though the polarizing effect as usually observed after the current has flowed for some time may be to decrease in amplitude or even or render or keep the nerve non-irritable. However, so far as can be observed in the visible potential record, it is impossible to obtain any depression of potential or change of form due to a current that ceases as the potential wave rises; i.e., involving polarization corresponding to the threshold. The applied potential must be very much greater than threshold to produce depression, and last over into the response.

Such changes in form as occur are due to the fact that different axons are stimulated at successive times, the summed potentials being those recorded.

7. *Repetitive response of nerve to the galvanic current.* When the action potential is made monophasic by killing under the anode, the bridge balance is restored at the end of the wave to the below-threshold position. If the applied potential is sufficiently high, the nerve may then respond again. Such a condition, in which several successive responses are evident, is shown in figure 8; the potential being led off as in figure 2. Since different sized fibers in the nerve have different thresholds (Gasser and Erlanger, 1927) and different utilization periods, the axon waves in the smaller fibers lag behind those in the larger fibers more and more in successive repetitive processes, prolonging the summed nerve potentials, until they finally overlap, and the picture becomes confused.

8. *Irritability after the first response to a galvanic current.* However, for the first three responses, at a lower strength of galvanic current, the irritability has been plotted as in figure 3. The shock-strength values required to produce a threshold response at various times preceding the waves are plotted in figure 9*A*, for the waves of the record of figure 8*A*. On the same figure are plotted the times of the crests of the waves of figure 8*B*. The interval apparently increases with the number of repetitions and the amplitude falls. However, since the waves increase in length as axon responses fall out of phase, the intervals between the *starts* of these waves will be approximately constant. With prolonged stimulation of this strength the nerve soon fails to respond repetitively. The accuracy of these measurements probably merits no more exact analysis (see legend).

When we consider the irritability relationships indicated in figures 3 and 9, it appears that the differences in the shape of the curve of return of irritability after response from the curve of the increase of irritability before response, may be connected with the condition of refractoriness from the last excitation. The irritability, as measured by shock strength, returns after a single response somewhat as when the nerve is being first

stimulated to threshold value at the beginning of current flow (fig. 9*a*). If this threshold state after a first response involves the re-attainment of a specific counter EMF of polarization, as might be inferred from the previous experiments, the further inference should be that the nerve recovers while refractory, during the passage of the current which previously polarized it, in order to respond again due to a *repetition* of the *polarization*. However, with stronger current, the curve of return of

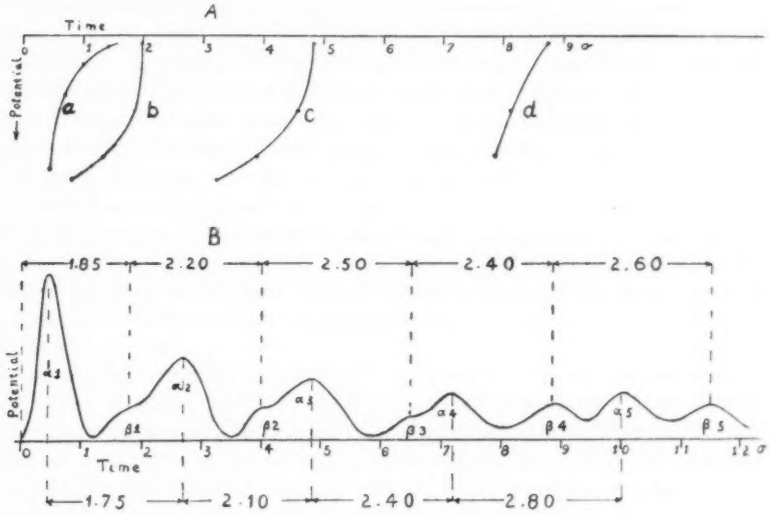


Fig. 9. A. Curve *a*, return of irritability, measured by strength of shock (below base line) necessary to stimulate after the single response to a weak galvanic current. Curves *b*, *c* and *d*, the same for a stronger current, sufficient to give three responses approximately as in figure 8 A.

B. Replot on linear coördinates of the responses of figure 8 B. There are apparently two series of responses, separating out more distinctly with time. One set has been labelled provisionally as the alpha component of the mixed nerve (α), the other as the beta (β). The waves tend to fuse as fibers of different characteristics fall out of phase with repetition, due presumably to differences in the current densities across the axon surfaces.

irritability takes on a different form, the curvature being in the opposite direction (fig. 9, *b*, *c*, *d*). Furthermore, the interval between successive waves, though approximately constant, bears no apparent relationship to the utilization period, which is the time, theoretically, necessary for threshold polarization. For while that of the first α wave is very short, and that for the first β wave about 0.001 second at this strength of current,

the intervals for the succeeding waves are approximately equal for the two fiber sizes. This equality is referable to the fact that the absolutely refractory phases of fibers of different sizes are about the same (Erlanger, Gasser and Bishop, 1927).

The second response may then be due not to a second polarization process, but to a recovery of the ability to react to the persistent polarization, a function which gives no sign of being associated with a change of resistance in the nerve. The nerve, being polarized continuously, responds whenever it becomes excitable, not after intermittent recovery from polarization, but after intermittent recovery from refractoriness. Absolute refractoriness is by definition merely the inability to respond due to a previous response; and while cathodal polarization decreases the potential response, and increases the duration of refractoriness (Bishop and Erlanger, 1926) the relation of these facts to a polarization counter EMF is not obvious. The action current, to be sure, opposes the stimulating current at the cathode, but a return of the potential to the status quo ante is even more prompt in cathodally polarized than in unpolarized nerve (Bishop and Erlanger, loc. cit.). The stimulating current, in a sense, may be continuously effective, though response to it has been prevented by what, for lack of information, we can merely describe as refractoriness.

Discussion. In the stimulation of nerve by any abruptly applied electric current, a characteristic polarization potential appears to be set up. Even with brief induction shocks, which are practically over before response, the action potential always rises before the polarization potential has subsided and presumably at or before its maximum. For induction shocks, the maximum of the polarization effect will fall later than the maximum of the applied potential, in fact, the fall of the applied potential curve should theoretically cut through the crest of the counter EMF curve. This crest or maximum of effect necessarily occurs when the two potential forces effective at the membranes are equal and opposite, after which the counter EMF discharges, and the remainder of the shock is effective only in slowing the discharge. The maximum of polarization, and hence threshold stimulus, is thus accomplished at some time during the fall of the summated effect, but is impossible to recognize as such in the record. Since the applied current is largely wasted in non-effective ways, the actual values of applied and counter potentials may be of quite a different order of magnitude (see Bishop, 1927, fig. 3, 1) and the polarization effect may not be particularly noticeable in a record made to the scale of the applied potential.

This immediate polarization effect associated with stimulation may reasonably be differentiated from the subsequent effects of further current flow, ordinarily dealt with under the head of polarization or electrotonus.

When galvanic currents of strength sufficient to cause the classical polarization effects are applied to nerve, the times necessary to bring about such effects are considerably longer than those here dealt with. Experiments analyzed above (section 6) describe the character of the first response to a galvanic current which lasts throughout the response. Even when considerably supermaximal stimuli are applied to nerve, action potentials recorded from the polarizing cathode are not appreciably different from those elicited by currents of the same strength but shorter duration, or by induction shocks. If such a current is allowed to flow longer, subsequent shocks elicit potential waves showing the typical cathodal reduction in amplitude. The same effect occurs in repetitive responses to strong galvanic stimuli ("closing tetanus" fig. 8). Only if the current is made very strong, does reduction of amplitude become appreciable in the first response to the galvanic stimulus. The two effects, immediate polarization accompanying stimulation, and gradual depression of function with time of current flow, are thus of different order of magnitude, and possibly of different type. The after-effects of current flow correspond. Summation of subthreshold stimuli in nerve is only effective if the second stimulus falls upon the polarization curve of the first, but the after-effects of long lasting polarization currents are persistent for long times. The natural inference is, that the immediate effect might be a simple membrane polarization, due to ions concentrated at an interface, whose charge and discharge resembles that of a condenser, while the persistent effect comprises a more persistent electrolysis, i.e., either the carrying of ions across the interface, or the deposit out of some ionic constituents at it, with a consequent change in the concentration or character of the reactants available for subsequent responses. Severe polarization may affect the staining reactions of the axons (Schwarzte, 1911) and may alter the nerve membranes (Schultze, 1926), (Woronzow, 1924, 1925). There appears to be no reason to conclude from these facts, however, that currents adequate to stimulate, do so by a lesser degree of the same type of effect.

The stimulation of nerve by an electric current is presumably to be explained upon the basis of the physicochemical changes that can be produced by current flow across any semipermeable system. The immediate changes are 1, concentration, at the interface, of ions whose movements are impeded by it; 2, electrodeposition of ions as molecules, and 3, oxidation (or reduction) of ions in solution due to electronic or ionic exchange across the membrane. The other alternative is the transport of ions across the membrane unchanged, which, in so far as it takes place, does not result directly in a polarization counter EMF, but acts like an ohmic resistance (permeability of the membrane). The third case is really a special case of 2, the essential difference being that in the case of electrodeposition, which is also an oxidation or a reduction, the deposited

layer becomes the effective electrode (as in the deposit of a metal from a solution of its salts). In such a case, above the potential necessary for deposition, further increase of current increases the polarization counter EMF but little, while in case 3, the counter EMF increases with concentration until all available material is converted to one form.

In passing current through such a structure as nerve, one might expect both concentration of ions and oxidation-reduction changes; and to what extent the membranes allow ions to pass through unchanged is unknown. Not only is it gratuitous to assume any profound structural change in the membranes due to the currents required to stimulate nerves, but such an assumption does not appear to explain electrical excitation, though such an alteration might be invoked to account for the phenomena of electrotonus. The significant fact here is that the above concentration changes *will* be associated with characteristic and transient and readily reversible polarization counter potentials; in fact, such potentials are a measure of these changes. If the concentration change is to be confined to a protein-salt solution complex, it is difficult to see why attainment of a specific polarization should result in nerve response. A counter EMF, however, that represented a shift in an oxidation-reduction system might set off chemical processes of a more "physiological" character, at a critical degree of concentration. Such an oxidation-reduction mechanism would give the same sort of polarization curve as does an ordinary semipermeable membrane. An electrode in a solution of reductant, for instance, may be polarized anodally, shifting its potential as the oxidized product increases in its surface, and discharging its potential as this product diffuses away or is otherwise disposed of.[3] Such a system does not seem to be a far-fetched analogy for nerve.

Thus in several respects the stimulation process may correspond to an essentially simple polarization phenomenon. At the same time deviations from a theoretically simple polarization process might be expected to result from the complexity of nerve as an electrolytically conducting system. Besides those deviations here considered, in the form of the time-strength threshold curve, stimulation by a "period of no current" and the effect of rate of change of potential appearing as the "minimal gradient" may also be susceptible of a physical interpretation on the basis of nerve reactance, an attempt at which will be made in a subsequent paper.

Such deviations from ultimate and obvious simplicity as the nerve affords, however, do not appear as serious obstacles to believing that up to the time of nerve response to abrupt electrical stimuli, nothing has

[3] Ellingham and Allmand (1924, p. 754) refer to an observation of Haber and Russ (1904) on the quinhydrone electrode. A definite polarization is set up before either oxidation or reduction takes place with even small current densities, and when the electrode is stirred.

happened of physiological importance except a local and transient concentration process, with a corresponding potential change, which in effect enhances the reactivity of some cell constituent. Failure of this enhanced reactivity to take effect in "response" appears to leave the nerve unaffected after the subsidence of the transient change of reactivity, which disappears *pari passu* with its potential sign.

SUMMARY

1. The hypothesis that polarization of some specific structure to a specific potential corresponds to the threshold of stimulation is presented graphically as a plot of Lapicque's formula. The lack of exact correspondence between this formula and experimental data is discussed in terms of the complexity of nerve as a conductor.

2. The change of irritability of nerve to an induction shock during a subthreshold galvanic current is plotted from cathode ray oscillograph data. This change approximates to an exponential function.

3. The polarization EMF curve of nerve is recorded directly by means of the oscillograph.

4. The change of irritability during a galvanic current follows approximately the same curve as does the polarization counter EMF.

5. The polarization curve changes with duration of galvanic current in a way which suggests two polarization effects of different order, possibly in different structures.

6. The differences in form of the responses to galvanic currents of different duration and intensity are described. These differences are consistent with the inference of two polarization effects of different character. The response starts with no appreciable latent period after just effective stimulation (within 0.000,02 second).

7. Repetitive responses of nerve to galvanic current stimuli are recorded close to the site of stimulation after balancing out the stimulating potential distortion in a Wheatstone bridge.

8. The return of irritability after successive responses to a galvanic current of long duration is plotted in terms of strength of superposed induction shock necessary for stimulation. Once polarized by the current, the nerve responds repetitively at a rate chiefly determined by its refractoriness, and does not appear to be depolarized during response. It thus appears to exhibit a true repetitive response to a *constant* stimulus, rather than a restimulation from a constant applied potential.

9. The irritability change which nerve undergoes due to electrical stimulation is a transient effect, subsiding with its potential sign, like a condenser charge. It may be differentiated from electrotonic effects of polarizing currents, which are of a different order of magnitude and duration.

BIBLIOGRAPHY

BISHOP, G. H. 1927. This Journal, lxxxii, 462.
BISHOP, G. H. AND J. ERLANGER. 1926. This Journal, lxxviii, 630.
BISHOP, G. H., J. ERLANGER AND H. S. GASSER. 1926. This Journal, lxxvi, 204.
ELLINGHAM, H. J. T. AND A. J. ALLMAND. 1924. Trans. Faraday Soc., xix, 748.
ERLANGER, J., H. S. GASSER AND G. H. BISHOP. 1927. This Journal, lxxxi, 473.
GASSER, H. S. AND J. ERLANGER. 1927. This Journal, lxxx, 522.
HABER, F. AND R. RUSS. 1904. Zeitschr. f. Physik. Chemie, xlvii, 257.
HILL, A. V. 1910. Journ. Physiol., xl, 190.
HOORWEG, L. 1892. Pflüger's Arch., lii, 87.
LAPICQUE, L. 1907. C. R. Soc. de Biol., lxii, 615.
1926. L'Excitabilite en Fonction du Temps. Les Presses Universitaires de France. Paris.
NERNST, W. 1904. Zeitschr. f. Electrochemie, xxv, 665.
SAMOIJLOFF, A. 1925. Pflüger's Arch., ccix, 484.
SCHULTZE, P. 1926. Pflüger's Arch., ccxi, 157.
SCHWARTZE, A. 1911. Pflüger's Arch., cxxxviii, 487.
VERZÁR, F. 1912. Zentralbl. f. Physiol., xxvi, 399.
WEISS, G. 1901. C. R. Soc. de Biol., liii, 466.
WORONZOW, D. S. 1924. Pflüger's Arch., cciii, 300.
1925. Pflüger's Arch., ccx, 672.

POLARIZING EFFECTS OF ELECTRICAL STIMULI UPON CONTRACTION OF SKELETAL MUSCLE

JOHN M. McCAUGHAN AND GEORGE H. BISHOP

From the Department of Physiology, Washington University School of Medicine, Saint Louis

Received for publication January 3, 1928

Results of certain student exercises of the course in physiology have shown that striated muscle commonly gives lower contractions with make induction shocks than with break shocks, even when both shocks are "maximal." It seemed possible that the make shock, lasting longer than the break, might stimulate different muscle fibers at different times, and thus render the summated maximal twitch longer in duration but lower; or else that some effect of current flow from the long lasting make shock reduced the power of the muscle. Records taken with a spring lever on a moving drum indicated that the durations of make and break twitches were approximately equal, when the contractions differed considerably in amplitude, both shocks being supramaximal. In these experiments the current was increased by steps to a value well above maximal and a reversing key in the secondary circuit allowed make and break shocks to pass through the muscle with the same polarity. The stimuli were equally spaced and the interval between stimuli sufficient to permit practically complete recovery (fig. 1).

The time at which the make shock of a Porter coil, arranged with a 50 ohm shunt across the primary terminals and 800 ohms in series with the secondary, falls to $\frac{1}{2}$ its maximal value is 10 times that for the corresponding break shock, as may be seen in photographs of the form of the induction shocks (fig. 2; also Erlanger and Garrey, 1914). The voltage of the make shock under these conditions can be increased to approximately that of the break if the primary current for the make be raised to a value of about 10 times that for the break. The consequent long duration of the current suggests that the maximal make shock might have a greater polarizing effect on the muscle, and thus in some way diminish its contraction.

The effect of current flow was tested on a bullfrog sartorius using the Lucas pendulum, but current from high constant potentials allowed to flow long enough to appreciably polarize the muscles often resulted in second responses. These parallel fibered muscles were stimulated near one

end, the anode being *at* the end. The greatest polarization would thus take place locally under the electrodes, only a small fraction of the contracting muscle substance being involved. Green frog gastrocnemii were therefore employed in the next experiments and the current passed length-

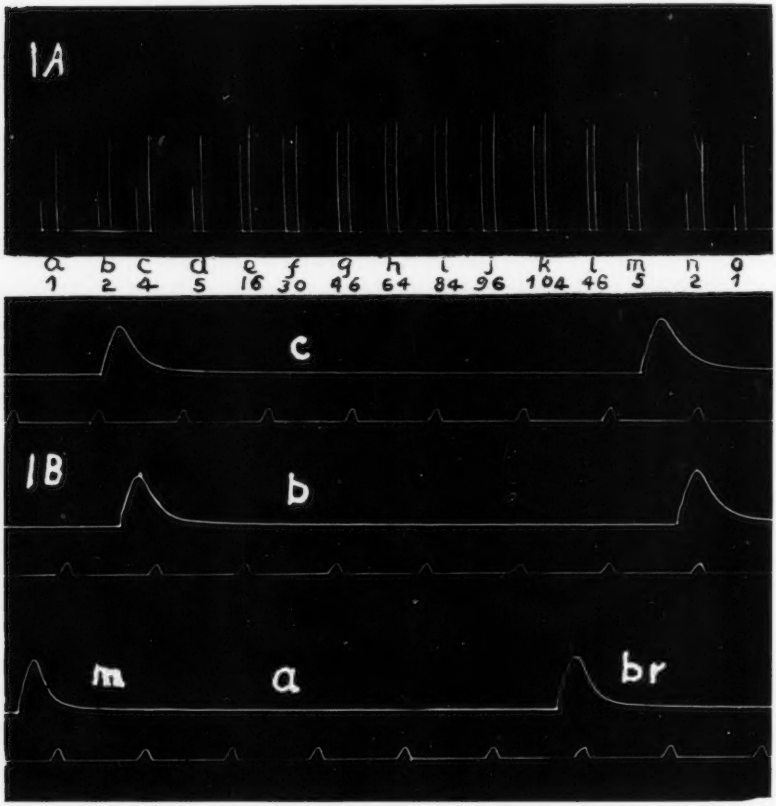


Fig. 1 A. Make and break shock responses with increase of current through primary, green frog gastrocnemius, isotonic (?) muscle lever record, against stretched rubber band as load. Strength of shocks increased from *a* to *k* in ratio *1* to *100*, *l m n o* same as *g d b a*, respectively.

1 B. Make and break shock responses of green frog gastrocnemius, spring lever. Strength of shock varied by slide wire in series with primary, when 90 cm. gave a maximal make shock. *a*. Make and break, slide wire 30 cm. current 2.5× that at 90 cm. which was apparently maximal. *b*. Slide wire 20 cm. current *3.2*× that at 90. *c*. Slide wire 7 cm. current *6*× that at 90. Time in 0.2 second. Break shocks responses are of constant amplitude. Make shock responses decrease with increasing stimulus.

wise through the whole muscle, but obliquely through each fiber. Condenser discharges were used as stimuli. By increasing the condenser capacity the duration of the stimulus is prolonged. A series showing the effect of varying the voltage was also taken. It will be seen from the accompanying figure (3) that beyond a certain voltage (maximum for the

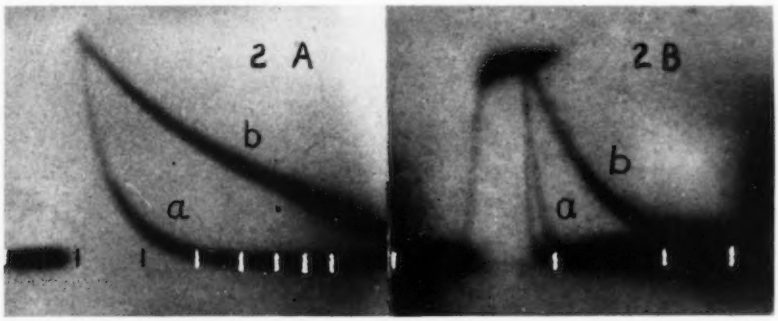


Fig. 2 A. Cathode ray oscillograph records of Porter coil shock potentials, *a*, break, and *b* make at about 10 × the recording sensitivity. Time in 0.001 second. For arrangement see text.

2 B. Cathode ray oscillograph records of condenser discharge *a*, 0.05 and *b*, 0.5 mf., through 1000 ohms for comparison with Porter coil shocks as muscle stimuli. The lowest curve gives a measure of the amplifier lag plus the capacity of the lead wires only. The make shock of 2 A corresponds to a 5.0 m.f. condenser.

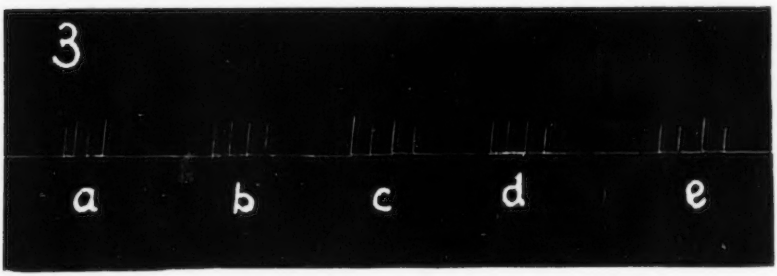


Fig. 3. Muscles responses to condenser discharges. Green frog gastrocnemius, spring lever. First and third, in each series of four are responses to discharges from a capacity of 0.05 microfarad, second and fourth 0.5 microfarad; voltages, $a = 40$, $b = 60$, $c = 80$, $d = 100$, $e = 120$. See figure 2 for form of discharge.

muscle) the heights of the contractions are lowered as more capacity is added. There is an optimum ratio of capacities for showing this effect. The bullfrog' sartorius stimulated near one end showed a similar phenomenon, but less markedly than the green frog gastrocnemius stimulated

end to end. The differences are more marked in a partially fatigued muscle, and vary from one muscle to the next.

The limits of capacity for condenser discharges which give the effects described lie between quantities too small to have appreciable effect in polarizing, and quantities so large that the muscle responds twice to one

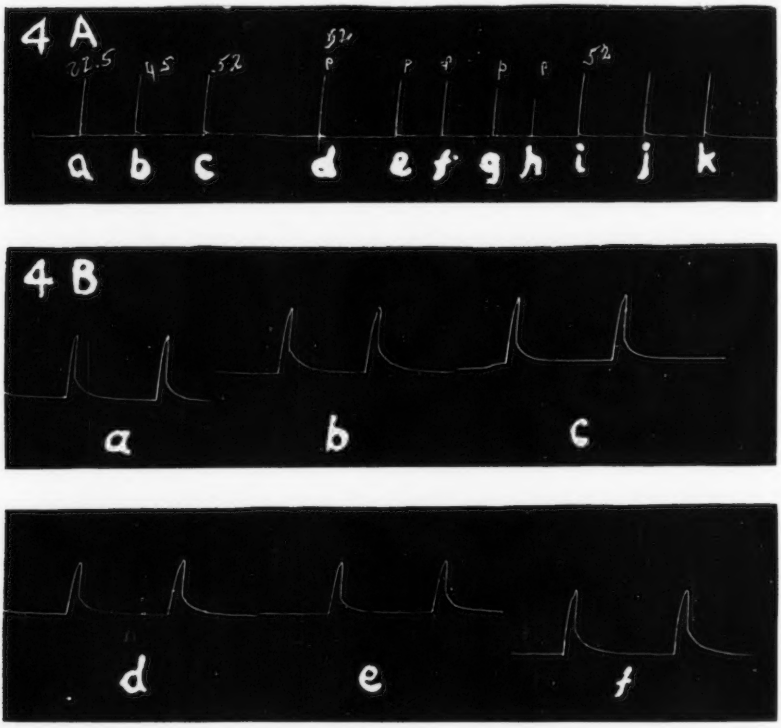


Fig. 4 A. Response of polarized green frog gastrocnemius muscle. *a*, unpolarized, stimulus 0.02 mf. capacity discharged from 22.5 volts; *b*, 45 volts; *c*, 52 volts; *d–h*, polarized by 3 volts through 13,000 to 1000 ohms, same stimulus as *c*; *i j k*, unpolarized recovery, same stimulus. Electrodes on ends of muscle, spring lever.

4 B. Moving drum records as above. Stimulus 0.02 mf. capacity 45 volts. *a*, unpolarized; *b*, polarized by 3 volts through 13,000 ohms; *c*, through 9000 ohms; *d*, 5000 ohms; *e*, 1000 ohms; *f*, unpolarized. Muscle approximately 1000 ohms.

discharge. As ordinarily employed, the Porter coil make shock with green frog gastrocnemius in the secondary circuit lies near the middle of this range, while the break shock falls outside it. The capacities will vary

widely with the condition of fatigue, etc., of the muscle. A pair of capacities may be found such that the response to any voltage from the larger condenser fails to reach the maximum elicited by discharges from the smaller, that is, some fibers may be depressed by a polarization effect before the less irritable have been stimulated at threshold. The same statement applies to make and break shocks.

In the next experiment a polarizing current was sent lengthwise through the muscle and a condenser discharge superposed through the same electrodes. In this case we used the gastrocnemius muscle because the direction of its fibers permits a more complete polarization. The results are indicated in figure 4. Comparison of the polarized and nonpolarized responses shows that polarization definitely decreases the height of contraction and the amplitude decreases as the strength of the polarizing current increases. We have not found a statement to this effect in the literature on muscle, though the result is to be expected by analogy with results on nerve (Bishop, Erlanger and Gasser, 1926; Bishop, 1928).

SUMMARY AND CONCLUSIONS

1. Polarization of striated muscle produces some change in the condition of the muscle which alters the power of its contraction.

2. Make shocks may result in contractions of less amplitude than break shocks because of the polarizing effect of the former.

3. This finding does not invalidate the all-or-none law if we consider that a stimulus, besides exciting, may also alter the state of the muscle contractile mechanism, before the response is accomplished.

BIBLIOGRAPHY

Bishop, G. H., J. Erlanger and H. S. Gasser. 1926. This Journal, lxxvi, 204.
Bishop, G. H. 1928. This Journal, lxxxii, 417.
Erlanger, J. and W. E. Garrey. 1914. This Journal, xxxv, 377.

THE EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT

V. The Offspring[1]

JAMES ROLLIN SLONAKER

From the Department of Physiology, Stanford University, California

Received for publication December 19, 1927

We have previously given the results of the effect of various amounts of sexual indulgence on growth, spontaneous activity, food consumption and the length of sexual life of the albino rat (Slonaker, 1927a, b, c; 1928). In the first of these papers we gave in detail the plan, method and procedure of the experiment. Only a mere summary, therefore, will be given here.

Our animals were divided into five groups and each group was permitted a predetermined amount of sexual indulgence. Group A consisted of pure virgins and strict bachelors which were never permitted any sexual intercourse. Group B was composed of light breeders whose sexual activities resulted in not less than two nor more than three litters during their life span. Group C consisted of medium breeders. This group produced and reared as many litters as possible, throughout life. This represented normal conditions as closely as we were able to make them. Group D were classed heavy breeders. In this group the young were destroyed at birth and the pairs remated as soon and as often as possible as long as they lived. Group E consisted of hysterectomized females and normal males which were kept constantly together. Since the removal of the uterus does not interfere in any way with the normal oestrual cycles these animals had excessive sexual indulgence without the possibility of bearing young. Five females from each of the groups A, B, C and D were confined throughout life in individual revolving cages to determine their spontaneous activity, normal oestrual cycles, gestation periods and pseudopregnancies. All other animals were kept in uniform stationary cages.

With the exception of the two types of cages used and the amounts of sexual activities permitted all the animals were subjected to as nearly as possible the same environment. They were all kept in the same well ventilated and lighted room in the same conditions of light, heat and mois-

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the Committee for Research on Sex Problems of the National Research Council.

TABLE 1

Giving the average size of each litter and average age of mother at delivery

Litter	1		2		3		4		5		6		7		8		9		10		11		12		13		14	
Group	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother	Size	Age mother
Revolving B	5.4	153	6.4	259	0.8	397																						
Stationary B	4.0	115	6.2	177	2.0	528																						
Average	4.8	136	6.3	223	1.4	456																						
Revolving C	11.4	171	9.4	218	6.8	273	5.6	327	5.2	416	3.0	487	2.0	526	1.8	540												
Stationary C	5.0	116	6.5	162	6.5	223	8.7	268	4.2	315	5.7	364	5.5	419	4.7	478	3.5	514	3.0	563	2.0	600	3.0	641				
Average	8.5	147	8.2	193	6.4	251	7.0	301	4.8	371	4.2	432	3.6	467	3.1	513	1.6	530	1.3	551	0.9	565	1.3	585				
Revolving D	10.0	146	9.6	187	9.4	234	4.8	271	5.6	301	4.6	344	2.2	370	0.6	404	0.2	420	0.4	427								
Stationary D	7.2	113	7.0	148	8.0	175	9.7	200	9.7	227	6.0	260	5.7	283	6.5	309	5.6	336	4.7	390	4.0	415	0.8	431	1.5	440	0.5	455
Average	9.0	131	8.4	170	8.8	208	6.7	239	7.4	268	5.2	307	3.8	331	3.2	362	2.7	383	2.3	411	1.8	422	0.3	429	0.6	433	0.2	440

ture, and were always fed more than they would consume between feeding times on the same well-balanced synthetic food. Any differences found in the results of the different groups have, therefore, been assumed to be caused by the different amounts of sexual indulgence these groups were permitted to have.

The results which follow were derived from groups B, C and D only, since they were the only groups which bore young. The experiment was started when the animals of these groups were at the average age of 76 days. Those in the revolving cages were slightly older than in the stationary cages, averaging 77 and 75 days respectively. All evidence indicates that none had reached sexual maturity at this age. Our activity records show that the females in the revolving cages reached sexual maturity at the average age of 89 days and by productive matings those in stationary cages became sexually mature at the average age of 92 days.

Size and number of litters. In table 1 we have given the average size of each litter and the average age of the mother at the birth of each litter. This shows that the first few litters in the revolving cages were larger than corresponding litters born in stationary cages and that the reverse obtained in later litters. The size of the successive litters in group B agrees with the results of other investigations in that the second litter was larger than the first and the last litter, born much later in life, was the smallest. In groups C and D, however, the first litters, which were born at nearly the same ages of the mothers as group B, were the largest and the successive litters gradually diminished in size. Forced breeding without intervals of rest appears to have caused a gradual reduction in the size of successive litters. It is also noted in groups C and D that the females in stationary cages threw a greater number of litters than those in the revolving cages. This was due to the fact that the mating tests of the revolving cage females were often non-productive due to not having been made at the critical time in oestrus. Many possible matings were therefore missed. In the stationary cages the sexes were constantly together and the chances for mating at the first opportunity were always present. In our previous paper (Slonaker, 1927d) we showed that the average precentages of true matings of the females in revolving cages, as indicated by the presence of sperm in the vaginal smears, which resulted in pseudopregnancy, were for the groups B, C and D, 43, 50 and 66 respectively.

Table 1 also shows that the average size of corresponding litters was least in group B and greatest in group D. But when we compare the size of the litters thrown by mothers of approximately corresponding ages we find that the medium breeders, group C, had, in general, the largest litters. It is further seen that the average age at delivery of the last litter was 145 days later in life in group C than in group D. As we have previously shown, interfering with normal reproduction (by killing the young at

TABLE 2

Giving the total and average weights at birth of each sex in each litter and the ratio of males per 100 females for each group of breeding females

GROUP	LITTER	REVOLVING CAGES					STATIONARY CAGES					TOTAL					TOTAL YOUNG		
		Male		Female			Male		Female			Male		Female					
		Number	Total weight	Number	Total weight	Sex ratio	Number	Total weight	Number	Total weight	Sex ratio	Number	Total weight	Number	Total weight	Sex ratio	Number	Total weight	Average weight
B	1	14	73	13	63	108	8	47	8	45	100	22	120	21	108	105	43	228	5.30
	2	17	88	15	72	113	11	62	14	73	79	28	150	29	145	97	57	295	5.17
	3	1	6	4	20	25	3	17	5	23	60	4	23	9	43	45	13	66	5.07
Total		32	167	32	160		22	127	27	141		54	294	59	301		113	595	
Average			5.22		5.00	100		5.77		5.22	81		5.46		5.10	91.5		5.26	
C	1	34	162	23	101	148	12	69	8	43	150	46	231	31	144	148	77	375	4.87
	2	22	109	25	110	88	12	67	14	66	86	34	176	39	176	92	73	352	4.82
	3	17	84	17	76	100	14	79	12	62	117	31	163	29	138	107	60	301	5.02
	4	11	61	17	79	65	16	82	19	97	84	27	143	36	176	75	63	319	5.07
	5	7	40	19	100	37	8	45	9	40	89	15	85	28	140	54	43	225	5.23
	6	6	31	9	43	67	10	47	13	56	77	16	78	22	98	73	38	176	4.63
	7	6	30	4	20	150	13	72	9	41	144	19	85	13	51	146	32	136	4.25
	8	1	6	3	16	33	8	40	11	52	73	9	62	14	78	75	23	140	6.08
	9						5	27	9	42	55	5	27	9	42	56	14	69	4.92
	10						7	46	5	21	140	7	46	5	21	140	12	67	5.58
	11						3	15	5	24	60	3	15	5	24	60	8	39	4.88
	12						6	32	6	27	100	6	32	6	27	100	12	59	4.91
Total		104	523	117	547		114	612	120	571		218	1135	237	1118		455	2253	
Average			5.03		4.67	89		5.36		4.76	95		5.20		4.72	92		4.95	
D	1	26	131	24	110	108	16	84	13	67	123	42	215	37	177	113	79	392	4.96
	2	24	120	24	117	100	13	60	15	65	87	37	180	39	182	95	76	362	4.76
	3	21	105	26	116	81	17	85	15	70	113	38	190	41	186	93	79	376	4.76
	4	12	53	12	53	100	19	96	20	79	95	31	149	32	132	97	63	281	4.46
	5	16	85	12	59	133	15	77	24	115	62	31	162	36	174	86	67	336	5.02
	6	10	53	13	69	77	13	65	11	53	118	23	118	24	122	96	47	240	5.11
	7	6	34	5	26	120	10	46	13	54	77	16	80	18	80	89	34	160	4.21
	8	1	6	2	11	50	14	70	12	56	117	15	76	14	67	107	29	143	4.93
	9			1	7	0	14	75	9	46	155	14	75	10	53	140	24	128	5.33
	10			2	11	0	7	35	12	59	58	7	35	14	70	50	21	105	5.00
	11						6	31	10	46	60	6	31	10	46	60	16	77	4.81
	12						1	5	2	9	50	1	5	2	9	50	3	14	4.67
	13						2	11	4	20	50	2	11	4	20	50	6	31	5.17
	14						1	5	1	5	100	1	5	1	5	100	2	10	5.00
Total		116	587	121	579		148	745	161	744		264	1332	282	1323		546	2655	
Average			5.06		4.79	96		5.03		4.62	92		5.04		4.70	93.6		4.85	

birth and remating at the earliest possible date) resulted in earlier sterility, accompanied by a slight increase in the average number of young and litters born. The explanation for the larger number of young in the D group was due to the fact that young mothers produce larger litters than old mothers.

In table 5 we have given the average number of litters, the average size of the litters and the average number of young delivered in groups B, C and D. The females in group B were permitted an average of 2.67 litters which averaged 4.7 young each. This low average was largely due to the last litters, averaging 1.4 young, being born late in life when the mothers were at the average age of 452 days. Sexual abstinence for the 223 days intervening between the second and the last litters seems to have influenced the productive ability of the female. The average size of the litter in group C at approximately the same age was 3.6 young. The average number of litters in group C was 8.66 and in group D it was 10. The greater number of litters in group D was expected since group C spent approximately 175 days of sexual life nursing the young. The average size of the litters in groups C and D was 5.83 and 6.83 young respectively. This difference can be explained by the fact that in group D all the young were born at a relatively early age. That is, in group D they were delivered between the average ages of 131 and 440 days while in group C the average ages between the first and last litters were 147 and 585 days. These results conform to the fact that litters born late in sexual life are usually small. The average length of sexual life for groups C and D was 460 and 331 days respectively.

Weight of young at birth. The weights of the young were ascertained as soon after birth as they were discovered. In some cases they were weighed immediately after delivery, but in the majority of instances a sufficient time intervened for the young to nurse. The weights gotten, therefore, were a trifle heavier than newborn rats. The weights at birth of each sex in the successive litters and the average weights are given in table 2. This shows that the males were heavier than the females in each of the groups. The per cent of increased weight of the male over the female was in group B 7.06, in C 10.02, in D 7.21. The average for the three groups was 8.13 per cent. All these differences in weight of the sexes are slightly in excess of that found by King (1915). Her results show that in the young delivered by mothers of various ages the males surpassed the females in weight by 6.53 per cent. The average weight of each in all three of our groups (table 2) was greater than that given by King. She found the males averaged 4.56 grams and the females 4.28 grams. Table 2 also shows that the young of group B were the heaviest, that those of group D were the smallest, and that those of group C were almost midway between the two extremes. The different amounts of sexual indulgence permitted these different groups was probably only indirectly the cause of the differences

in weight of the young at birth. It is generally known that the young of small litters have a heavier birth weight than those of large litters. The average size of the litters in group B (see table 5) was 4.7 which was the smallest of any of the groups. This would favor the increase of weight of the young. In group D, whose young had the lowest birth weight, we find the largest litters. This would tend to make their young smaller. In group C the average size of the litter was about midway between B and D. A direct correlation between weight of young at birth and size of litter is thus established.

Weight of young at weaning. Table 3 gives the average ages and weights of each sex and the total young of groups B and C at the time of weaning. Since all the young of group D were destroyed after weighing at birth only the light and medium breeders can be compared in regard to the weights of the young at weaning. The above table shows that the young of group B were slightly older and noticeably heavier than those of the C group. The difference of 0.55 of a day in age was not sufficient to account for the differ-

TABLE 3

Giving the average ages and weights of each sex and total averages for the young of groups B and C at weaning

GROUP	MALES			FEMALES			TOTAL YOUNG		
	Number	Average age	Average weight	Number	Average age	Average weight	Number	Average age	Average weight
B	33	23.15	35.68	32	23.15	35.4	65	23.15	35.5
C	136	22.6	33.7	149	22.6	30.3	285	22.6	31.9

ence in weight. At this age our rats gain almost 2 grams daily. The difference in age would, therefore, only account for about 1 gram. The explanation for the greater weight of the young from light breeders in our opinion was not due so much to the differences in sexual indulgence as to more favorable conditions for nourishment in the light breeders than in the medium breeders. That is, the young of group B were not only heavier at birth, but also, due to the litters being smaller, received a more copious supply of milk during the nursing period than the young of group C. The weights of each sex in both groups were considerably greater than given by Donaldson (1924) averaging about 34 per cent greater in group B and 21 per cent in group C.

Mortality and cannibalism. In table 4 we have given the number and per cent of each sex which died and the number and per cent which were eaten before weaning in the successive litters of groups B and C. In group D, since the young were destroyed at birth, we have given only the number and per cent of stillborn and those which died before weighing. The per

TABLE 4

Giving the number of each sex born and the number and per cent of each sex which died before weaning and were eaten in each litter

The percentages are based on the number of each sex born. All D young were destroyed at birth.

GROUP	LITTER	REVOLVING CAGES										STATIONARY CAGES										TOTAL										TOTAL YOUNG				
		Male					Female					Male					Female					Male					Female									
		Number	Died	Per cent	Eaten	Per cent	Number	Died	Per cent	Eaten	Per cent	Number	Died	Per cent	Eaten	Per cent	Number	Died	Per cent	Eaten	Per cent	Number	Died	Per cent	Eaten	Per cent	Number	Died	Per cent	Eaten	Per cent	Number	Died	Per cent	Eaten	Per cent
B	1	14	1	7	1	7	13	3	23	3	23	8	3	37	3	37	8	3	37	3	37	22	4	18	4	18	21	6	28	6	28	43	10	23	10	23
	2	17	8	47	8	47	15	9	60	9	60	11	4	36	2	18	14	3	21	2	14	28	12	43	10	36	29	12	41	11	38	57	24	42	21	42
	3	1					4	1	25	1	25	3	1				5	5	100	5	100	4	1	25			9	6	67	6	67	13	7	54	6	46
Total		32	9		9		32	13		13		22	8		5		27	11		10		54	17		14		59	24		23		113	41		37	
Average				28		28			41		41			36		23			41		37			31		26			41		39			36		33
C	1	34	5	15	5	15	23					12	1	83			8					46	6	13	5	11	31					77	6	8	5	7
	2	22	15	68	15	68	25	18	72	18	72	12					14					34	15	43	15	43	39	18	46	18	46	73	33	45	33	45
	3	17	9	53	9	53	17	10	59	10	59	14	1	71	1	71	12	1	83	1	83	31	10	32	10	32	29	11	38	11	38	60	21	35	21	35
	4	11	6	54	6	54	17	4	23	4	23	16	7	44	7	44	19	5	26	5	26	27	13	48	13	48	36	9	25	9	25	63	22	35	22	35
	5	7	5	71	5	71	19	13	68	13	68	8					9	4	44	4	44	15	5	33	5	33	28	17	61	17	61	43	22	51	22	51
	6	6	4	67	4	67	9	4	44	4	44	10	3	30	1	10	13	7	54	5	38	16	7	43	5	31	22	11	50	9	41	38	18	47	14	37
	7	6	3	50	3	50	4	2	50	2	50	13					9	2	22	2	22	19	3	16	3	16	13	4	31	4	31	32	7	22	7	22
	8	1	1	100	1	100	3	3	100	3	100	8					11	2	18	2	18	9	1	11	1	11	14	5	36	5	36	23	6	26	6	26
	9											5	1	20	1	20	9	4	44	2	44	5	1	20	1	20	9	4	44	2	22	14	5	36	3	21
	10											7	4	57	4	57	5	1	20	1	20	7	4	57	4	57	5	1	20	1	20	12	5	42	5	42
	11											3	2	67	2	67	5	2	40	2	40	3	2	67	2	67	5	2	40	2	40	8	4	50	4	50
	12											6					6	2	33	2	33	6					6	2	33	2	33	12	2	17	2	17
Total		104	48		48		117	54		54		114	19		16		120	30		26		218	67		64		237	84		80		455	151		144	
Average				46		46			52		52			17		14			25		22			31		29			35		34			33		32
D																																				
Total		116	3				121	1				148	3				161	1				264	6				282	2				546	8			
Average				2.58					0.83					2.02					0.62					2.36					0.71					1.47		

cents are based on the sex born except in the column of total young, where they represent the percent of the total young. It is noticed that the per cent of mortality in the B and C groups was greatest in the second litter and least in the last one. This table also shows that the total mortality of these two groups was nearly the same being only slightly greater in the B group (36 per cent) than in the C group (33 per cent). The mortality of the males was 31 per cent in each of these groups and that of the females was 41 per cent in group B and 35 per cent in group C. This shows that up to the weaning age the mortality of the females was greater than that of the males in both these groups. In group D, however, the number of males which were stillborn or died before they were weighed at birth was three times that of the females. This shows that the vitality of the male is less than that of the female during prenatal life and agrees in general with the results of other investigators (King, 1921; Lewis and Lewis, 1906; Hirsch, 1913; Davis, 1920). The total per cent of stillbirths

TABLE 5

Giving the average length of gestation, number of litters, size of litters, weight of each sex, sex ratio, the per cents of total mortality of young and young eaten up to the weaning age of each group of breeding females

GROUP	AVERAGE GESTATION PERIOD		AVERAGE NUMBER OF LITTERS	AVERAGE SIZE OF LITTER	AVERAGE TOTAL YOUNG	AVERAGE WEIGHT		NUMBER OF MALES TO 100 FEMALES	PER CENT OF TOTAL YOUNG DIED	PER CENT OF TOTAL YOUNG EATEN
	Days	Hours				Male	Female			
B	21	20.8	2.67	4.7	12.6	5.44	5.1	92.0	36.00	33
C	22	2.2	8.66	5.83	50.6	5.20	4.72	92.0	33.00	32
D	22	6.0	10.00	6.83	60.7	5.05	4.69	93.6	1.47	

for group D is very close to that given by other investigators. The different amounts of sexual indulgence in these three groups apparently had little or no effect on the mortality of the offspring.

Cannibalism is very common in the rat. It is not only the young which are eaten, but also mature rats have been found partly devoured. In a former paper (Slonaker, 1923) we found in a large group of rats consisting of 1239 young whose mothers were fed on a diet consisting largely of table scraps supplemented with cracked yellow corn that there was a mortality of 36.4 per cent and that 26.8 per cent of the total young born were eaten. This amounted to 73.6 per cent of those which died. Table 4 shows that in groups B and C of this experiment the number of total young eaten was 33 and 32 per cent respectively. The number of those which died that were eaten was 90 per cent in group B and 95 per cent in group C. The per cent of mortality of the young in this experiment was slightly less and the per cent of cannibalism was greater than in the former experiment. The results cannot be attributed to differences in sexual indulgence since

the diets in the two experiments were different. The very slight differences in the mortality and in cannibalism in groups B and C indicate that the amount of sexual indulgence permitted each of these groups had little or no effect in this respect.

Length of gestation. In table 5 we have given the average length of the period of gestation in the three breeding groups. This shows that in the light breeders, group B, the gestation period was the shortest and in the heavy breeders, group D, it was the longest. Group C was almost the mean of the other two groups. In a former paper (Slonaker, 1925) we showed that mothers carrying large litters had a shorter gestation period than those carrying small litters. This was especially true with young females. In litters with an average size of 2.67 the average gestation period was 22 days 5.5 hours. The range in size was from one young with a gestation period of 23 days and 5 hours to three young having a gestation of 21 days 22 hours. In litters with an average size of 4.83 (range 4 to 6) the average time of gestation was 21 days 22.9 hours. Litters of the average size of 8 (range 7 to 9) had an average gestation duration of 21 days 14 hours. In the largest litters which averaged 11 young (range 10 to 14) the average gestation period was 21 days 4.78 hours. The shortest period of gestation was 21 days in the largest litter (14) and the longest period was 23 days 5 hours in the litter of one young. We also noted a tendency for the gestation period to lengthen with increasing age of the female. Those animals were subjected to only a medium amount of breeding. The average gestation period of 49 pregnancies representing different litter sizes and ages of mothers was 21 days 17 hours. In this experiment one female in group B which delivered its first litter of 10 young at the age of 126 days had a gestation period of 20 days 6 hours. The tendency of a lengthening of the gestation period with advancing age is also noted. The reduction in litter size, the increase in age and the lengthening of the gestation period seem to be directly correlated. The results of this experiment show a direct opposite to those of the previous investigation. That is, group D, which had the greatest number of litters and the largest litters, had the longest period of gestation and group B, which had the fewest and smallest litters, had the shortest gestation period. The gestation period of group B was almost the same as that found in our previous investigation. The lengthening of the gestation period in groups C and D we attribute to the forced sexual indulgence. Normal delivery apparently is induced when the young have reached a certain stage of intra-uterine development. This we find requires normally 21 days 17 to 20 hours. If the vitality of the mother should be reduced it is very probable that the intra-uterine development of the young to the stage of delivery would be delayed, thus lengthening the time of gestation. The almost continuous bearing and rearing of young in the C group apparently

had this retarding effect. The killing of the young at birth and the delivery of a greater number of litters and young in group D apparently had a still greater retarding effect on intra-uterine growth. This is also in accordance with the lower birth weights of the young in the C and D groups.

The ratio of the number of males to the number of females born (table 5) is much lower than the normal of about 106 males to 100 females found by other investigators. This low sex ratio was found in all groups of breeding animals and we are unable to give an explanation.

SUMMARY

Our results show that when albino rats are subjected to various amounts of sexual indulgence the offspring is affected in the following ways:

1. In the group of light breeders the size of the successive litters conformed to the normal. That is, the second litter was larger than the first and the last litter was the smallest. In groups of continuous breeding and of heavy breeding the first litter was the largest and successive litters were gradually smaller.

2. Mothers of a given age which nursed their young had larger litters than mothers of like age whose young had been continually destroyed at birth.

3. The medium breeders which reared as many litters as possible were productive for 145 days later in life than heavy breeders whose young were destroyed at birth. The latter, however, delivered a greater number of litters and young.

4. The interference of normal reproduction by killing the young at birth and remating at once resulted in sterility of the female at the average age of 440 days. Sterility occurred in the females of medium breeders at the average age of 585 days.

5. The average length of sexual life of the medium breeders was 460 days and of the heavy breeders 331 days.

6. In all groups larger litters were thrown by mothers in the early part of sexual life than in the later part. This conforms with other investigations.

7. The cessation of reproductive activities for 223 days resulted in smaller litters (group B) than when reproductive activities had been continuous.

8. The weight of the young at birth was heaviest in the light breeding group and lightest in the heavy breeding group.

9. The weight of the young at birth was in direct correlation with the size of the litter.

10. The males surpassed the females in weight at birth in all groups by a slightly greater per cent than found by other investigators.

11. The weight of the young at weaning was greater in the light breeders than in the medium breeders. This is in direct correlation with litter size.

12. In groups B and C the per cent of mortality was greatest in the second litter and least in the last litter.

13. Mortality of the young was slightly greater in the light breeders than in the medium breeders.

14. In groups B and C the mortality of the males was less than that of the females.

15. The number of males greatly surpassed that of the females in the stillborn and those which died at birth in group D.

16. Evidence indicates that the male has a less vitality than the female before birth and a greater vitality than the female after birth.

17. Cannibalism was only 1 per cent greater in group B than in group C.

18. The average lengths of gestation in groups B, C and D were 21 days 20.8 hours, 22 days 2.2 hours, and 22 days 6 hours respectively.

19. Former experiments showed a direct correlation between a reduction in litter size, advancing age and lengthening of the gestation period. The results of this experiment are in direct opposition.

20. The lengthening of the gestation period in medium breeders and a still greater lengthening in heavy breeders is attributed to a reduced vitality of the mother, resulting in slower development of the intra-uterine young.

21. The sex ratio of all groups was below normal.

BIBLIOGRAPHY

DAVIS, W. H. 1920. Birth statistics for the birth registration area of the United States, 1918. Bureau of Census, Washington.

DONALDSON, H. H. 1924. The rat. 2d Ed. Memoirs Wistar Inst. Anat. and Biol., no. 6, 276.

HIRSCH, M. 1913. Centralbl. f. Gynäkol., xxxvii.

KING, H. D. 1915. Anat. Rec., ix, 213.
1921. Anat. Rec., xx, 321.

LEWIS, C. J. AND J. N. LEWIS. 1906. Natality and fecundity. New York.

SLONAKER, J. R. 1923. This Journal, lxiv, 297.
1925. This Journal, lxxi, 362.
1927a. This Journal, lxxxii, 318.
1927b. This Journal, lxxxii, 376.
1927c. This Journal, lxxxiii, 302.
1928. This Journal, lxxxiv, 192.

STUDIES ON THE KNEE JERK

VI. An Additional Note on the Effect of Raised Intrapulmonic Pressure on the Knee Jerk, Heart Rate and State of Consciousness

A. B. LUCKHARDT and C. A. JOHNSON[1]

From the Department of Physiology, University of Chicago

Received for publication December 24, 1927

Since the publication of a previous paper of this series on "The Effect of Raised Intrapulmonic Pressure upon the Knee Jerk, Arterial Blood Pressure and State of Consciousness" by Johnson and Luckhardt (1), it occurred to the authors to repeat some of the work upon animals under more normal conditions, i.e., animals which were not under the influence of any anesthetic.

With this in view preliminary experiments were done, and although the results upon the knee jerk were striking, equally striking results were had upon the heart rate and state of consciousness; and it is these three phenomena we wish to report on at this time.

Methods. Unanesthetized dogs were used provided with a tracheotomy and with lifting ligatures under both vagi. These preparations were made under surgical anesthesia (ether) six hours previous to any experiment in order that the animals might recover sufficiently from the operation and the anesthesia.

Upon such animals heart rate, respiration and the presence or absence of the knee jerk were observed consecutively during: 1, a control period; 2, a period of raised intrapulmonic pressure (which was produced by inflating lungs through the trachea provided with rubber tubing and clamping off the latter); 3, and for some time after the release from the raised intrapulmonic pressure. These observations were repeated after the intravenous injection of atropine and also after double vagotomy.

Because of the marked cardiac effects observed under these conditions the observations were repeated on anesthetized animals from which accurate records of heart rate, carotid blood pressure and knee jerk were recorded on a rapidly moving drum. Knee jerks were quantitatively measured by means of a special knee jerk apparatus described by one of us in a previous paper (2).

[1] This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

RESULTS. The results were as follows: The animals were induced to stand erect on a table. The dogs were usually a bit apprehensive but otherwise normal. The intrapulmonic pressure was then suddenly raised and maintained as previously described. In ten to fifteen seconds the dog would fall over unconscious with the legs in marked extensor rigidity and with the head in a position of opisthotonos. The position closely resembled the typical decerebrate rigidity of Sherrington. Defecation

TABLE 1

Showing records of the heart rate of unanesthetized dogs before, during and for some time after an experiment on raised intrapulmonic pressure

(Before and after double vagotomy or atropinization)

PREPARATION OF ANIMAL	PROCEDURE	TRIAL	PULSE DURING CONTROL PERIOD	R I P*		RECOVERY FROM R I P		
				Time of	Pulse during	Pulse immediately after	After release	
							Time	Pulse
Tracheotomy under ether 6 hours previous to experiment		1	108	60″	60	60	5′	60
							10′	60
							15′	60
							20′	60
	5 mgm. atropine intravenously	2	210	5′	204	204	15′	204
		3	204	4′ 30″	246	Death		
Tracheotomy and both vagi isolated under ether 6 hours previous to experiment	Vagi intact	1	120	2′ 44″			3′	48
							8′	72
							15′	90
							18′	96
	Vagi intact	2	96	4′	54		1′	60
	Vagi cut	3	282	5′	276	282		

* R I P = Raised Intrapulmonic Pressure.

and urination commonly occurred soon after. In time the animal became perfectly flaccid and seemed perfectly anesthetized, i.e., it seemed to be asleep and made no signs of perceiving painful stimuli following incisions of the skin. Even without sensory stimulation, no spontaneous movements of the animal were observed. After a single experiment lasting a few minutes the animals usually remained permanently on their side, but if given fifteen to twenty minutes for recovery would occasionally attempt to rise. In a few instances the animals got up and walked about. During

the period of flaccidity, the corneal reflex was present. As judged by approaching the finger to the eye, the animal was unconscious of visual impulses. The pupils were widely dilated. At this time it was found that the pulse was remarkably slowed (table 1 and fig. 1) and the knee jerk was quite "exaggerated" and other reflexes such as crossed extension, flexion and tail reflexes could often be elicited with ease. If the intrapulmonic pressure was maintained, the knee jerk and other reflexes now disappeared and if this was allowed to happen the animal could only be

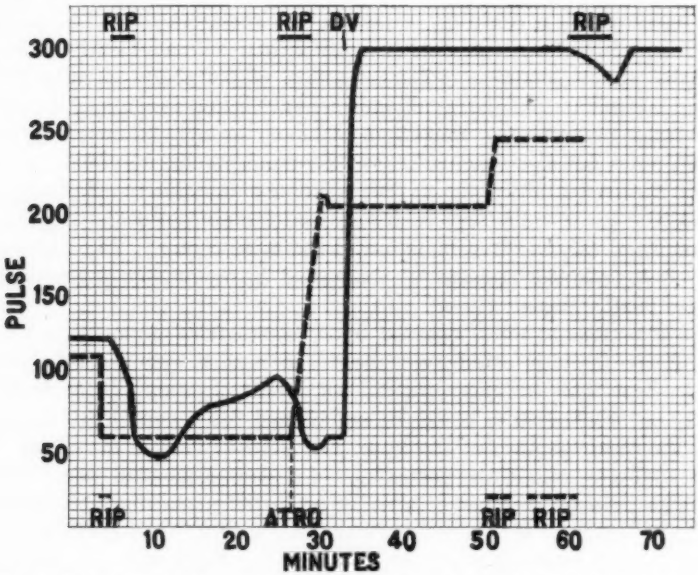


Fig. 1. Chart illustrating graphically the effect of raised intrapulmonic pressure on the heart rate of two unanesthetized dogs before and after atropinization or double vagotomy. *R I P*—raised intrapulmonic pressure; *A T R O*—atropine injected intravenously; *D V*—double vagotomy.

Record of one dog in continuous line; the other in broken line.

revived with difficulty, if at all. The length of time of raised intrapulmonic pressure necessary for the complete abolition of the knee jerk varied, but in some cases was as low as one-half minute. It might be added that it was impossible to revive one animal after maintaining raised intrapulmonic pressure for one-half minute. On release of the raised intrapulmonic pressure (if the animal survived) the reflexes returned rather promptly but the pulse rate returned to normal much more slowly (table 1 and fig. 1). In one case, as shown in table 1, the pulse dropped from a

normal of one hundred and eight per minute to sixty per minute and remained at sixty per minute for twenty minutes following release of the raised intrapulmonic pressure, at which time atropine was injected.

If in these animals atropine was given or double vagotomy was done and the experiment repeated, it was found that the heart rate could not be appreciably affected and in order to abolish the reflexes it was practically necessary to kill the animal by asphyxia, i.e., it was necessary to maintain raised intrapulmonic pressure for five to seven minutes. It should be understood that the raised intrapulmonic pressure as practiced by us practically causes a cessation of peripheral circulation (3). The experiments on atropinized and doubly vagotomized animals clearly indicate that the diminution to complete cessation of peripheral circulation on raising and maintaining intrapulmonic pressure is due not only to mechanical interference with the circulation of blood through the markedly distended lungs but also (particularly in the unanesthetized animals) to the central cardiac inhibition due, at the beginning of inflation to reflex stimulation of the cardio-inhibitory center and later maintained and intensified by asphyxial (chemical) stimulation of the same center. The prolonged after-effect following release of the raised intrapulmonic pressure was marked in all animals.

The experiments were repeated upon anesthetized animals on which quantitative records could be made. The results were essentially the same except that the knee jerk did *not* persist close up till the time of death, i.e., the pressure could be maintained for a considerable period after the disappearance of the knee jerk without danger of killing the animal. Furthermore, the persistence of a slow pulse after raised intrapulmonic pressure was not so striking as in the unanesthetized animals (table 2). Apparently the cardio-inhibitory center is less susceptible to direct and reflex stimulation if depressed by an anesthetic. Observations on the carotid pressure during raised intrapulmonic pressure in the anesthetized animal revealed that the arterial blood pressure dropped practically to 0 mm. Hg, as previously reported. In the unanesthetized animal the general blood pressure was not reduced so markedly even with the more or less marked accompanying cardiac inhibition; for the femoral pulse was obliterated only with difficulty and disappeared completely about the time of impending death. We attribute this difference to the greater efficiency of the vasoconstrictor center of the unanesthetized animal.

Discussion. The interpretation of the phenomena in view of these experiments and results previously reported by Johnson and Luckhardt (1) suggests the extreme importance of the reflex factor as well as the importance of the profound circulatory disturbance resulting in an anemia of the brain as well as an anemia of the peripheral structures in the unanesthetized animal (dog or man).

It also illustrates the difficulties and errors in interpretation one is liable to experience in drawing conclusions from observations made solely upon animals under an anesthetic. In studying the effect of raised intrapulmonic pressure upon the knee jerk in the anesthetized dog we did

TABLE 2

Showing records of the heart rate of anesthetized dogs before, during and for some time after an experiment on raised intrapulmonic pressure

(Before and after atropinization or double vagotomy)

PREPARATION of ANIMAL	PROCEDURE	TRIAL	PULSE DURING CONTROL PERIOD	RIP		RECOVERY FROM RIP		
				Time of	Pulse during	Pulse immediately after	After release	
							Time	Pulse
Paraldehyde 1.5 cc. per kilogram		1	204	20″	150	129	1′ 15″	139
							4′	161
Anesthesia moderately deep		2	161	29″	129	117	6′	161
		3	161	30″	117	117	4′	161
		4	168	60″	104	77	1′	132
							2′	144
Tracheotomy and both vagi isolated							5′	150
							7′	168
		5	168	1′ 15″	96	78	1′	114
							3′	144
	Atropine 5 mgm. intravenously	6	180	1′ 15″	168	150	1′	180
	Vagi cut	7	180	1′ 15″	192		1′	204
16 kgm. dog.		1	204	40″	96		1′	102
4 grams barbital-Na							2′	156
							4′	192
Tracheotomy and both vagi isolated		2	204	40″	102		2′	204
		3	204	50″	102	150	4′	222
		4	222	40″	108	165		
	Vagi cut	5	282	40″	282	282		

not consider the disappearance of the knee jerk as a danger signal; for it was possible in some cases to abolish the knee jerk by this procedure for three or four minutes. In the unanesthetized animal (vagi intact) the situation is different, i.e., the knee jerk persists almost to the point of

death, and in all cases (fourteen animals) it was difficult to revive the animal if the procedure was carried to the point of disappearance of the knee jerk. But in these experiments, the central nervous system, specifically the spinal cord, was not already depressed by an anesthetic.

In reviewing the literature on the cardio-vascular changes occurring as a result of inducing raised intrapulmonic pressure by the Valsalva experiment or various modifications of it, contradictory results were found with respect to both the changes observed and the interpretation thereof.

Philip Knoll (3) showed two tracings indicating an increased heart rate in both Valsalva's and Müller's experiments. We were only able to confirm this result when the intrapulmonic pressure was raised moderately, i.e., by straining with the glottis closed. When the pressure was raised suddenly and to a considerable degree we never noticed any primary increase in heart rate either in anesthetized or unanesthetized animals, suggesting that in moderate straining such as occurs in everyday life, there is a primary stimulation of the cardio-accelerator center to compensate for the increased output of energy while on the other hand by increasing the pressure, primary cardiac inhibition is the predominant change. The fact that distention of the lungs produces a reflex apnea is evidence that there is marked stimulation of the vagus endings and there is no reason why reflex cardiac inhibition cannot occur by this same mechanism. The additional evidence that after atropinization or double vagotomy, it is not possible to get a marked slowing also suggests that the predominant mechanism concerned in the slowing observed is a primary reflex cardiac inhibition with secondary asphyxial stimulation of the cardio-inhibitory center and not due to mere mechanical obstruction to cardiac action. Further, if the reflex mechanism is depressed by an anesthetic the results of raised intrapulmonic pressure are not so striking and this to us is considered evidence favoring the importance of the reflex factor in causing the marked slowing observed.

With respect to blood pressure findings during Valsalva experiments or its various modifications Dawson and Hodges (4) report a primary increase in blood pressure in human subjects of from 18 to 31 mm. of mercury and in only one case (PCH) was a secondary fall noted of 15 mm. of mercury and this fall only lasted two to fifteen seconds after the beginning of the experiment. As previously reported in a paper of this series (1), the authors in a modified form of Valsalva's experiment were able, on human subjects, to cause a primary drop in blood pressure of 50 mm. of mercury with a secondary rise of 24 mm. of mercury above normal, but in no case either in the human subjects or in the dogs did we ever notice a primary rise in blood pressure.

Dawson and Hodges performed several experiments on dogs studying the effects of raising the intrapulmonic pressure on the blood pressure.

The technic which we used subsequently was identical with their own. It would seem, however, that we employed a greater intrapulmonic pressure since our effects are more profound.

It is of interest to note that raising the intrapulmonic pressure by the Valsalva experiment or modifications thereof has been employed clinically in cases of tachycardia in an attempt to reduce the heart rate. Galli (5) mentions a case of tachycardia in which he was able to reduce the heart rate from 210 to 60 but for how long he does not say, but a tracing which he shows seems to indicate a gradual return to normal.

Mosler and Burg (6) report that in the normal individual raising the intrapulmonic pressure an average drop of twenty-four in the heart rate, followed by an increase and return to normal is observed. In the cardiac patients similar results were had but of a milder nature. They attributed the decrease in heart rate as due to the primary elevation of the blood pressure which supposedly effects stimulation of the depressor nerves. As the pressure falls, the depressor effect ceases and cardiac acceleration ensues. He thinks the procedure is dangerous in patients with a weakened heart. As previously stated, we are not inclined to the view that the slowing of the heart rate as observed is primarily due to an elevated blood pressure; for the slowing, in our experience, accompanies the profound drop in blood pressure. We feel that the main factor is stimulation of the cardio-inhibitory center through reflex stimulation of sensory nerves in the cardiac or pulmonary vagus, and the stimulation of the cardio-inhibitory center directly by asphyxia.

CONCLUSIONS

1. Increase in the intrapulmonic pressure of the unanesthetized dog markedly slows the heart and for a prolonged time by stimulating the center reflexly and as a result of direct central asphyxia; for this cardiac inhibition can be either prevented by a previous atropinization or by a previous double vagotomy. Similar qualitative results are observed in the dog anesthetized by suitable doses of barbital Na. The changes are, however, not so profound.

2. Marked increase in the intrapulmonic pressure of the unanesthetized dog renders the dog unconscious and anesthetic in 12 to 15 seconds. A semi-unconscious state persists for some time after.

3. Abolition of the knee jerk by raising the intrapulmonic pressure is not effected as readily in the unanesthetized as in the anesthetized dog presumably because of a greater difficulty in suspending the peripheral circulation in the unanesthetized animal by the experimental procedure. As a matter of fact the knee jerk disappears just prior to death. Resuscitation of the animal after its disappearance is difficult or impossible.

BIBLIOGRAPHY

(1) JOHNSON AND LUCKHARDT. 1928. This Journal, lxxxiv, 612.
(2) JOHNSON. 1927. This Journal, lxxxii, 75.
(3) KNOLL, P. 1894. Pflüger's Arch., lvii, 406.
(4) DAWSON AND HODGES. 1920. This Journal, l, 481.
(5) GALLI. 1924-25. Zeitschr. klin. Med., ci, 221.
(6) MOSLER AND BURG. 1925. Klin. Wochenschr., iv, 2238.

SOME EFFECTS OF EARLY CASTRATION ON THE GROWTH OF THE MALE RAT

GERTRUDE VAN WAGENEN

From the Department of Anatomy, University of California

Received for publication November 21, 1927

Stotsenburg (1909) seems to have been the first to make a systematic study of the growth after castration of males among mammals, using the albino rat. His conclusion that "in the case of the albino rats, the growth curve for castrates is similar to that of the normals" is widely quoted and assumed in texts and in investigations with allied interests (Donaldson and Hatai, 1911; Hatai, 1913; Steinach, 1912; Livingston, 1916; Moore, 1919; Donaldson, 1915, 1924; Lipschutz, 1924.)

The data presented in this paper do not negate the conclusions of Stotsenburg for the growth period he studied, that of the young rat. However in carrying observations beyond the 149, 181 and 185 days of his three series to 350 days in series A and 450 days in series B in this study, it becomes evident that the weight curves diverge and the earlier and decided plateauing of the castrates is significant.

Stotsenburg's weight graphs are compiled from rich data. Series 1 of 40 animals (21 castrated) was weighed throughout a period of 143 days at the University of Chicago in 1905; series 2 of 27 animals (14 castrated) and series 3 of 32 animals (17 castrated) were observed at the Wistar Institute, Philadelphia, in 1907 and 1908 respectively.

Donaldson and Hatai (1911), using the white rat, accepted Stotsenburg's conclusion as to "body growth" although in their tables there is evidence that the castrates were of shorter body length. Hatai (1913) stated that his observations supported Stotsenburg; for the growth of body in weight was not modified by castration, nor was the normal relation between body weight and body length modified, but the tail tended to be slightly longer in the castrate. The body length and tail length appear to have been recorded only at the time of killing. In a later (1915) study on the growth of organs as affected by gonadectomy he found the body length at killing to be 4 per cent in favor of the controls.

Steinach (1912) stated that when brother littermates had the same weight at four months a difference no greater than 15 grams in favor of the normals developed in the next four months. He said that therefore

in rat males the growth curves of normal and castrate were completely similar. In this case the animals were operated after sexual maturity.

Livingston (1916) working on rabbits described his curves as showing "practically no overgrowth of the castrated animals in body weight."

It is believed by Moore (1922) that the relative weights of guinea pigs are worthless as indicators of sexual condition. In his figure the weight curve for the castrated guinea pigs lies below that of the controls after the first ninety days. The final weights of the two groups at 350 days averaged 872 grams and 810 grams respectively and the final lengths 30.4 cm. and 28.6 cm. Measurements from which the latter averages were computed were made after killing.

According to Sumulong (1924) the castration of guinea pigs causes a moderate acceleration of growth in body weight, length, height and circumference.

Over 50 per cent difference in favor of the normal animal was found by Fish (1925) between the body weight of kids, two castrated and one control.

Evans and Simpson (1927) in experiments effecting gigantism in the white rat stated that among the untreated animals the gonadectomized male was lighter than his control. This agrees with my own findings (van Wagenen, 1927) according to which the animal is not only lighter but shorter in body length.

MATERIAL AND METHODS. *Series A.* For this study of castrated and normal male rats two series of animals were used. Series A, consisting of ten operated animals with their 7 littermate brother controls, was followed for 350 days during 1923–24. At weaning when the animals were 21 days of age the first weighings were recorded. In the operated rats the testes were removed through an abdominal incision while the animals were under ether anesthesia. In every case the epididymis was removed with the gonad; and the spermatic artery with its accompanying plexus of veins and the vas deferens were separately ligatured. The second weighings were made when the animals were 25 days old and the animals were weighed every five days thereafter throughout the experiment. Operated and control animals were kept together under the same laboratory conditions. Weighings were made at the same time each day in the hope of avoiding maximum variation of enteric contents. Distribution of individuals of this series is given in table 1.

Series B. Of the twenty-three males in the second series 12 were castrated and eleven littermate brothers were used as controls (see table 2). Eleven litters were represented and the animals chosen from each were those most nearly of the same weight. These first weights were recorded and the operations done the day after weaning when the animals were twenty-two days old, and in this series linear measurements were also made. Just before each operation, when the animal was thoroughly relaxed under

deep ether anesthesia, measurements from nose to anus and anus to tail tip (checked by total length) were made. After castration of the operated animals, the controls were placed under anesthesia and measured. Realizing the many possible sources of error, the routine of measuring was early established, and measurements were taken so frequently in this laboratory throughout a period of two years that it is hoped a certain accuracy was developed. Routine of measuring could not be hurried. With the animal lying on its back from left to right before the operator and the ether cone held in the left hand, the right hand was free to gently straighten the body and draw out the tail. Careful note of the last remaining reflexes following this action (i.e., a slight curving or shortening of the tail) and the time

TABLE 1

	LITTER NUMBERS	NUMBER OF INDIVIDUALS		
		Controls in each	Castrated in each	Totals
Growth series A 1923–1924	1, 2, 3	1	1	6
	4, 5, 6	1	2	9
	7	1	1	2
Totals		7	10	17

TABLE 2

	LITTER NUMBERS	NUMBER OF INDIVIDUALS		
		Controls in each	Castrated in each	Totals
Growth series B 1925–1926	1, 2, 3, 4	1	1	8
	5	1	2	3
	6, 7, 8, 9, 10, 11	1	1	12
Totals	11	11	12	23

they ceased made it possible to bring the animal to somewhat the same depth of anesthesia, at each time of measurement. When measuring the controls the testes were pushed up into the abdominal cavity relieving the tension on the skin around the anus and allowing it to take the same position in relation to the base of the tail as that found in the castrates.

Weights of all animals in series B were taken every five days beginning with day twenty-five and lengths were recorded every ten days from day thirty on for each animal throughout the experiment which ended when 450 days of age had been reached.

Discussion. In series A weights alone were recorded. Table 3 gives the individual weights in grams on the day of weaning and the weights of

the same animals at 350 days of age. On this later date, in every instance, the castrated brother weighs considerably less than its control, the minimum difference within a litter being −14 per cent and the maximum −38 per cent. The weight of each control was taken as 100 per cent and the castrate compared accordingly. The average per cent difference is −25.8.

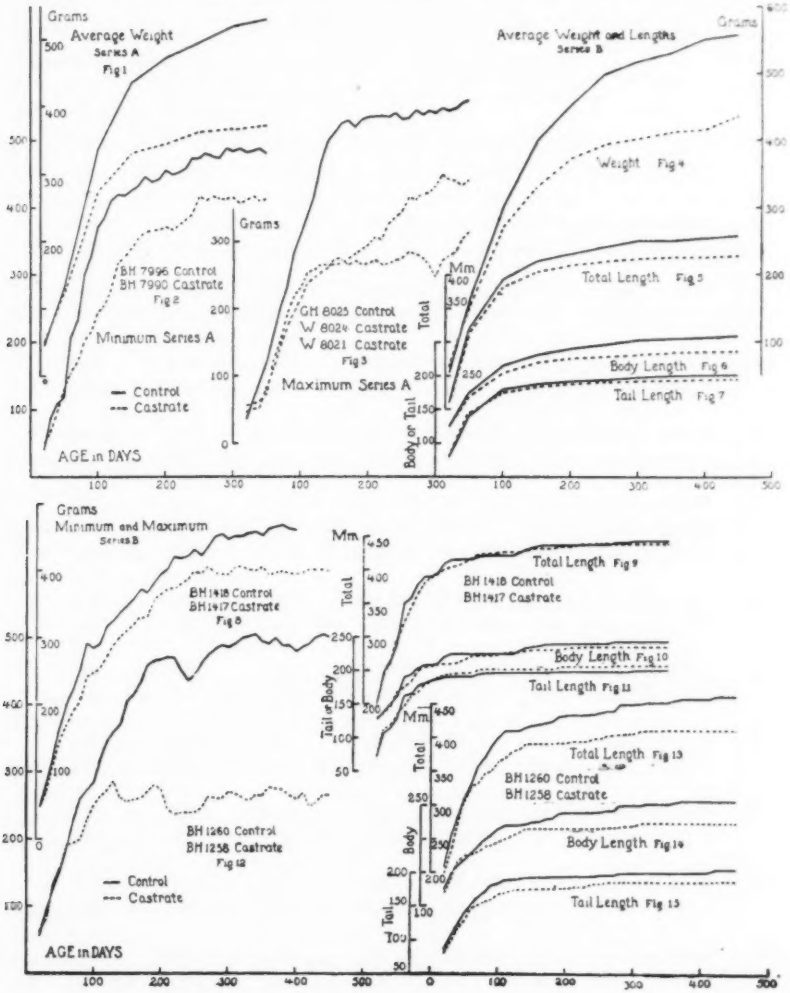
TABLE 3

Weights for series A

LITTER NUMBER	ANIMAL	21 DAYS	350 DAYS	CASTRATE DIFFERENCE EXPRESSED AS PER CENT OF CONTROL
		grams	*grams*	
1	BH7980 Cont.	46	558	
	W7978 Cast.	52	352	−37
2	B7983 Cont.	40	492	
	B7982 Cast.	44	391	−21
3	BH7996 Cont.	41	481	
	BH7990 Cast.	50	412	−14
4	B8005 Cont.	49	572	
	BH8002 Cast.	59	376	−34
	BH8001 Cast.	53	287	−16
5	GH8010 Cont.	41	512	
	BH8006 Cast.	45	412	−20
	BH8006 Cast.	45	344	−33
6	GH8025 Cont.	36	510	
	W8024 Cast.	56	390	−24
	W8021 Cast.	46	314	−38
7	B8037 Cont.	42	568	
	B8036 Cast.	49	450	−21
Average per cent difference				−25.8

Figure 2 presents the weight curves for the castrate and control showing the minimum difference in this series. These curves are based on observed weighings at ten day intervals. In figure 3 is shown the maximum difference found within a litter. Here two operated animals are compared with a control, the greater discrepancy in final weights (196 grams) occurs between GH8025, control, and W8021, castrate. The temporary depression in growth rate of both castrates immediately after operation was found in no other case.

Average weight curves for the two groups of operated and unoperated animals in this series are given in figure 1. Observed weights for the individuals of each group were averaged at 21 days, 50 days and at 50-day



intervals following. No considerable difference is shown during the first 100 days but the decided and earlier plateauing of the curve representing the operated animals results in an increasing difference in weight which reaches ultimately the value of 160 grams.

In series B lengths (body, tail and total) and weight were followed throughout a period represented in the age of the animals from 22 days to

TABLE 4

Weight and lengths—series B

LITTER NUMBER	ANIMAL	22 DAYS (TIME OF OPERATION)				450 DAYS (DIFFERENCE IN CASTRATE EXPRESSED AS PER CENT OF ITS CONTROL)							
		Weight	Body	Tail	Total	Weight	Per cent	Body	Per cent	Tail	Per cent	Total	Per cent
		gm.	*mm.*	*mm.*	*mm.*	*gm.*		*mm.*		*mm.*		*mm.*	
1	B1041 Cont.	54	126	83	209	470		258		210		468	
	BH1042 Cast.	60	123	82	205	352	−25	230	−11	189	−10	419	−10
2	W1051 Cont.	55	129	80	209	494		248		192		440	
	BH1052 Cast.	58	128	71	199	522	+6	242	−2	177	−8	419	−5
3	BH1072 Cont.	56	127	77	204	636		254		210		464	
	W1073 Cast.	59	129	80	209	450	−29	241	−5	213	+1	454	−2
4	W1182 Cont.	54	127	79	206	500		273		194		467	
	W1185 Cast.	54	131	84	215	450	−10	245	−10	193	−1	438	−6
5	W1214 Cont.	53	127	82	209	561		257		207		464	
	W1216 Cast.	54	125	85	210	513	−9	240	−7	205	−1	445	−4
	W1215 Cast.	52	125	83	208	468	−17	230	−11	187	−10	417	−10
6	GH1227 Cont.	54	132	71	203	590		268		206		474	
	GH1229 Cast.	57	128	76	204	557	−6	245	−9	195	−5	440	−7
7	BH1260 Cont.	56	123	85	208	509		256		205		461	
	BH1258 Cast.	60	118	80	198	264	−47	223	−13	187	−9	410	−11
8	W1286 Cont.	60	124	84	208	591		263		201		464	
	W1287 Cast.	58	118	86	204	390	−34	236	−10	190	−5	426	−8
9	W1305 Cont.	56	132	85	217	661		253		196		449	
	W1306 Cast.	60	136	84	220	550	−17	234	−8	197	+1	431	−4
10	W1312 Cont.	52	120	72	192	642		247		184		431	
	W1310 Cast.	51	118	82	200	350	−45	230	−7	191	+4	421	−2
11	BH1418 Cont.	48	128	73	201	467		243		200		443	
	BH1417 Cast.	50	130	71	201	398	−15	236	−3	207	+4	443	0
Average per cent difference of castrate							−20.7		−8.0		−3.3		−5.8

450 days. Weighings were made every 5 days and length measurements every 10 days.

Table 4 gives the observed data for the day at the beginning and the day at ending of the experiment. In the latter case there is shown in addition the per cent by which the various castrate measurements differ from the corresponding control measurements. The significant data here seem to be the weight and body length. The tail length does not consistently respond to the operation and this in turn influences the total length. The average difference of castrate weight is −20.7 per cent and of body length is −8.0 per cent.

Weight and length curves for individuals of the litters showing greatest and least differences are given in figures 8 to 15. Average weight and length curves for the two groups in series B (figs. 4 to 7) again show the failure of the castrates to reach the control in weight and body length.

Recognition of these effects is important in endocrine experimentation, e.g., it may be that certain effects attributed to ovaries, which have been implanted in the castrated male, may have been the result rather of the absence of the excised glands.

CONCLUSIONS

Male albino rats which have been castrated at the time of weaning do not maintain the weight and body length of their unoperated control brothers.

This failure is not always evident during the first 100 to 150 days but after that time there is an increasing divergence between curves for weight and between curves for body length.

Body length rather than total length is the significant linear measurement.

BIBLIOGRAPHY

DONALDSON, H. M. 1924. The rat. Memoirs of the Wistar Institute of Anatomy and Biology, vi.

DONALDSON, H. H. AND S. HATAI. 1911. Journ. Comp. Neurol., xxi, 417.

EVANS, H. M. AND M. E. SIMPSON. 1927. Anat. Record, xxv, 36.

FISH, P. A. 1925. Proc. Soc. Exper. Biol. and Med., xxii, 248.

HATAI, S. 1913a. Amer. Journ. of Anat. xxv, i, 87.

1913b. Journ. Exper. Zoöl., xv, iii, 297.

1915. Journ. Exper. Zoöl., xviii, i, 1.

LIPSCHUTZ, A. 1924. The internal secretions of the sex glands.

LIVINGSTON, A. E. 1916. This Journal, xl, 153.

MOORE, C. R. 1919. Journ. Exper. Zoöl., xxviii, 137, 459.

1922. Biol. Bull., xliii, 285.

STOTSENBURG, J. M. 1909. Anat. Record, iii, 233.

SUMULONG, M. D. 1924. Philippine Journ. Sci., xxvii, 325.

VAN WAGENEN, G. 1927. Anat. Record, xxxv, 51.

GROWTH RESPONSE TO ANTERIOR HYPOPHYSEAL EXTRACT BY THE CASTRATED MALE RAT

GERTRUDE VAN WAGENEN

From the Department of Anatomy, University of California

Received for publication November 21, 1927

If an extract of the anterior lobe of the hypophysis is injected intraperitoneally in albino rats a gigantism is effected (Evans and Long, 1921; Evans and Simpson, 1927). The object of this study was to test the efficacy of this substance in the gonadectomized male rat. It was thought that length measurements made at ten-day intervals during the period of treatment would give valuable data concerning body growth in addition to the usual weight records.

The extract used was made from beef glands according to the method of Evans and Long (1921). Ten litters were chosen of three or four brothers each. Only litters within which the variation was less than 8 grams, on the day of weaning, were used. The animals least in weight were selected for injection. These did not always prove to be the shortest in length at first measurement, but differences were slight.

The thirty-two animals from ten litters were grouped in the following way: 1, twelve animals castrated at twenty-one days to be injected with extract of the anterior lobe of the hypophysis; 2, ten animals castrated at the same age to remain uninjected; 3, ten normal controls.

The injected animals received intraperitoneally 1 cc. of fresh extract daily. For all animals the weights were recorded at five day intervals and every ten days measurements of nose to anus and anus to tail tip (checked by total length) were made under deep ether anesthesia.

The accompanying table presents the data for each animal at close of the experiment when the animals were four hundred and fifty days old. For comparison within each litter the control measurement was used as one hundred per cent and the castrate or castrate-injected difference was given accordingly a plus or minus value.

Those rats from which the testes were removed at the time of weaning failed to attain the weight or body length of their control brothers. The average difference in weight was −24 per cent and in body length −7.9 per cent. Although the average per cent difference in tail length was −3.1 per cent the response to the operation was not consistent, and in three out of ten cases the tail length was greater than that of the control,

TABLE 1

Weight and length at 450 days

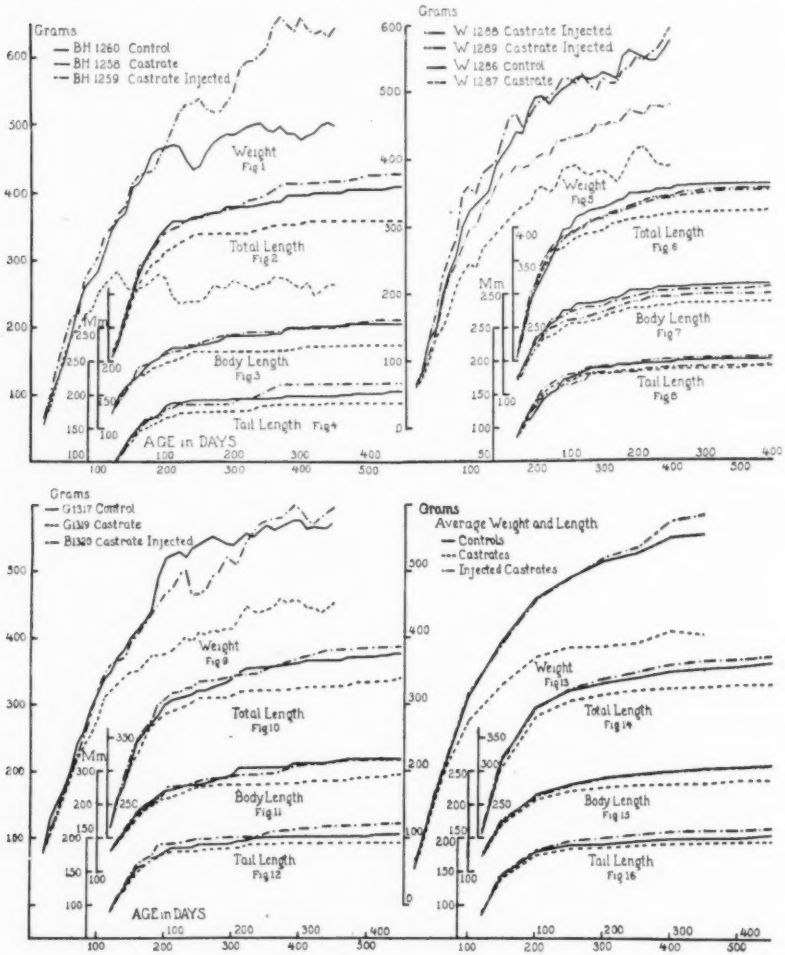
For comparison within each litter the control measurement was used as 100 per cent.

	WEIGHT	PER CENT DIFFERENCE	BODY	PER CENT DIFFERENCE	TAIL	PER CENT DIFFERENCE	TOTAL	PER CENT DIFFERENCE
	grams		*mm.*		*mm.*		*mm.*	
W192 Cont.	566		258		207		465	
GH1297 Cast. inj.	583	+3	252	−2	218	+5	470	+1
W1296 Cast.	459	−19	241	−7	208	0	449	−3
W1305 Cont.	633		246		193		439	
W1308 Cast. inj.	692	+9	268	+9	222	+15	490	+11
W1309 Cast. inj.	461	−27	251	+2	208	+8	459	+5
W1306 Cast.	352	−44	235	−4	196	+2	431	−2
BH1072 Cont.	642		254		209		463	
W1074 Cast. inj.	*304*		252	−1	223	+7	475	+3
W1073 Cast.	434	−32	241	−5	213	+2	454	−2
W1051 Cont.	526		248		193		441	
W1055 Cast. inj.	*390*		246	−1	193	0	439	0
BH1052 Cast.	524	0	239	−4	177	−8	416	−6
BH1260 Cont.	500		256		200		456	
BH1259 Cast. inj.	646	+29	258	+1	217	+9	475	+4
BH1258 Cast.	265	−47	223	−13	186	−7	409	−10
BH1120 Cont.	519		260		193		453	
BH1123 Cast. inj.	580	+12	255	−2	223	+16	478	+6
BH1122 Cast.	498	−4	240	−8	192	−1	432	−5
BH1041 Cont.	420		258		212		470	
BH1040 Cast. inj.	634	+51	250	−3	210	−1	460	−2
BH1042 Cast.	352	−16	230	−11	189	−11	419	−11
W1312 Cont.	636		247		186		433	
W1311 Cast. inj.	476	−25	242	−2	206	+11	448	+3
W1310 Cast.	*276*		230	−7	190	+2	420	−3
W1286 Cont.	578		263		201		464	
W1288 Cast. inj.	594	+3	260	−1	194	−3	454	−2
W1289 Cast. inj.	481	−17	251	−5	205	+2	456	−2
W1287 Cast.	390	−33	236	−10	191	−5	428	−8
G1317 Cont.	578		268		204		472	
B1320 Cast. inj.	595	+3	265	−1	220	+8	485	+3
G1319 Cast.	455	−21	241	−10	193	−5	434	−8
Average per cent difference:								
Cast. inj.		+5.1		−0.1		+6.4		+2.5
Cast.		−24.0		−7.9		−3.1		−5.8

Note: Italicized weights had fallen precipitously during the preceding fifty days so were not used in computing averages.

which meant a relatively longer tail because of the shorter body length of castrated males.

The deficiency in weight and lengths of the castrated group was not observed in the group of castrates which had been injected. In figures



1 to 12, which give the curves for the individuals of three litters, it will be seen that the curves for the injected castrates lie with or above the control curves. However, the curve for body length always follows closely the control.

Curves for the litter most susceptible to the growth stimulation of the anterior hypophyseal substance are shown in figures 1 to 4. The body weight of the injected castrate follows that of the control and rises considerably above it in the latter half of his life. The curve for body length of the injected castrate lies with the control. The total length curve for the injected castrate lies above the like curve for its control, but this is due, for the most part, to the greater response in tail length. Figures 5 to 8 present the curves for the litter least influenced. The results, while less striking, are of the same character. Group averages in weight and lengths, plotted in figures 13 to 16, seem to warrant the following conclusions.

CONCLUSIONS

The body length, tail length, total length and weight of the untreated male castrate is less than like data for the control and injected castrate.

Injection of anterior hypophyseal extract raised the body length of the injected castrate to that of the control and the weight of the injected castrate, in most cases, considerably above that of the control. Body weight in respect to body length is greater in the hypophyseally treated castrate than in the control.

Although the body length of the control and injected castrate is about the same, the total length of the injected castrate is greater because of the definitely greater response in tail length.

BIBLIOGRAPHY

EVANS, H. M. AND J. A. LONG. 1921. Anat. Record, xxi, 62.

EVANS, H. M. AND M. E. SIMPSON. 1926. Anat. Record, xxxii, 206.

1927. Anat. Record, xxxv, 35, 36.

SMITH, P. E. AND T. P. SMITH. 1922. Proc. Soc. Exper. Biol. and Med., xx, 51.

SMITH, P. E. 1927. This Journal, lxxx, i, 114.

VAN WAGENEN, G. 1927. Anat. Record, xxxv, 51.

THE STIMULATING ACTION OF HYDROSTATIC PRESSURE ON CARDIAC FUNCTION[1]

D. J. EDWARDS AND McKEEN CATTELL

From the Department of Physiology, Cornell University Medical College, New York City

Received for publication December 21, 1927

During a study of the influence of localized pressure upon nerve conduction (Cattell and Edwards, 1926) a question of practical and theoretical importance arose concerning the possible effects of uniform compression upon the functional activity of a tissue.

It is well known that pressure acting upon a short length of nerve trunk, or a strip of muscle tissue as in the classical "bridge experiment" of Gaskell, and the "A-V bundle clamp" of Erlanger, delays or completely blocks conduction of the impulse through the compressed region. But it is not clear whether this action is due entirely to the effects of differential pressure with the accompanying deformation of the boundary regions between compressed and non-compressed part, or whether uniform compression acting on all boundaries, as occurs probably to some extent in the center of a compressed region, may also play a part in depressing the property of conduction in a tissue that is under pressure.

Some observations have been made upon the effects of compression on physiological processes, particularly with extreme pressures and comparatively simple types of function. Chlopin and Tammann (1903) have shown that certain bacteria withstand enormous pressures without destroying them, and Hite (1920) reports that pressures of 100,000 pounds per square inch are required to inhibit, or to destroy enzyme action. Regnard (1891) working with frogs' muscles exposed for two minutes to a pressure of 300 atmospheres, observed a lengthening of the latent period, a decrease in the number of stimuli necessary for tetanus, and a prolongation of the curve of contraction; changes that are suggestive of the condition of fatigue. At a pressure of 100 atmospheres no changes were observed in the response of the muscle to electrical stimulation, and at 200 atmospheres the contractions were somewhat weakened.

[1] A preliminary summary of this work appeared in the Proc. Soc. Exper. Biol. and Med., 1927, xxv, 234.

We wish to acknowledge our indebtedness to the Director of the Marine Biological Laboratories at Woods Hole, Mass., for placing at our disposal the facilities for carrying out part of this work.

Tschermak (1902) describes a device by which the contractions of two closely related areas of a muscle may be compared while one is allowed to act freely and the other is compressed by loads of chosen amounts. His results show that the working capacity of a compressed area is much augmented, and that within limits, the increase in contractile response bears a direct relation to the magnitude of the compression force. Moreover, the local increased capacity for work under compression appears to have no influence upon adjoining non-compressed regions. The mechanism of this augmenting influence of pressure is not evident in Tschermak's results, but the conditions of his experiments do not preclude the possibility that increase in fiber length may have played an essential part. There is also the possibility that a certain degree of compression facilitates the distribution of the stimulus, resulting in the simultaneous action of a greater number of fibers.

These results suggest a kind of stimulating, or facilitating action of pressure upon muscular activity, but v. Uxkell (1895) concludes from his studies on nerve, that pressure is not a stimulus for this tissue, and Fredericq (1913) studying the relative effects of mechanical pressure simultaneously applied to muscle and nerve has shown that the function of conduction is abolished first in nerve.

The studies of Ebbecke (1914) have furnished important evidence in regard to the influence of compression upon muscle. Employing a special chamber constructed so as to admit a frog's muscle preparation and to give records of its contractions by means of an electrometric method, he investigated the effects on the muscle of pressures ranging from 100 to 600 atmospheres. Compression for short periods between the range of 200 to 300 atmospheres he concludes is without effect; whereas, in the pressure range from 300 to 400 atmospheres strong contractions usually appear but the muscle shows no evidence of injury since repeated compression elicits contraction of the muscle similar in character to those shown by the initial compression. On the other hand, if pressure between the 300 and 400 atmosphere range is maintained on the muscle for some time, changes occur which finally result in death of the tissue. By carefully graduating the onset of pressure and observing in terms of atmospheres the point at which contractions appear, the author determines for different muscle preparations, a value which he terms the "threshold stimulation in atmospheres." This value is not essentially different in curarized muscle so the foregoing observations of Ebbecke indicate that pressure may act as a stimulus for muscle independently of its nerve supply, and that muscle can withstand a compression of over 100 atmospheres without injury.

Gaertner (1920) has shown also that mice may be subjected to pressures up to 25 atmospheres for considerable periods without lethal effects. Moreover, it appears to be immaterial whether the compression is introduced suddenly, or is allowed to act in a gradual manner.

In the experiments to be described we have studied the influence on the heart of hydrostatic pressure so applied as to act uniformly on all surfaces of the organ. It has been necessary to devise a method which provides a means of subjecting the entire heart to the uniform compression, and simultaneously to record its contractile responses.

METHOD. We have employed, as a compression chamber for the heart, a thick walled metal bomb formerly used in calorimeter experiments and shown in figure *1*, *A*. This bomb is about 6 cm. in diameter and 12 cm. in depth, and is equipped with a thick walled head (fig. 1, *B*) which screws on firmly, and is practically free of leaks at 80 atmospheres, the highest

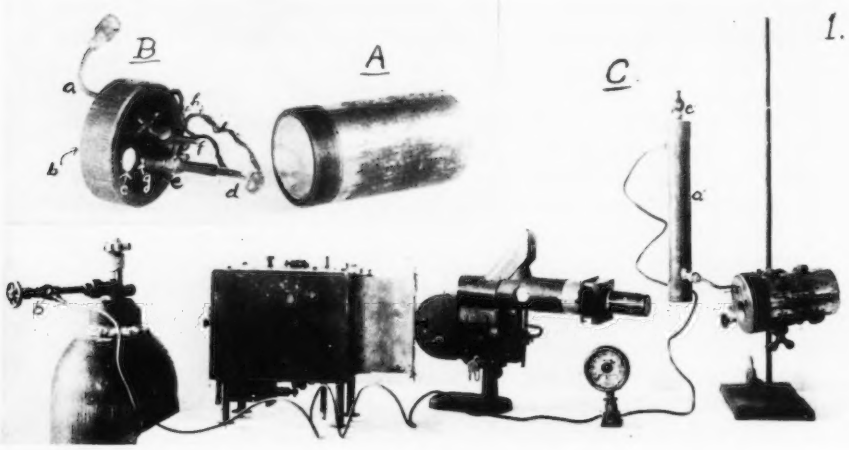


Fig. 1. Compression chamber and assembly for applying hydrostatic pressure to the heart. Described in the text.

pressure used in these experiments. The essential parts on the head are designated as follows; *a*, the tubing for connection to the source of pressure; *b*, two insulated binding posts for connections within the chamber; and *c*, a thick glass window through which records can be obtained of the contractions of the heart.

The heart preparations used were from the frog, the turtle, and the dogfish. In most instances the heart *d* was isolated, the veins were tied close to the sinus leaving the auricles to continue their rhythmic activity, and a small cannula was introduced into the aorta so that its tip projected past the valves and into the ventricle. A small segment capsule membrane manometer shown at *e*, figure 1, *B*, is attached to this cannula by a thick walled rubber tubing, and special care is exercised to have the system including the heart, the cannula, and the manometer entirely filled with Ringer's solution

free of air bubbles. The manometer with the heart attached, *d*, is clamped to the staff, *f*, and is arranged so that the small mirror, *g*, affixed to the rubber membrane, occupies a position just behind the glass window. The wires, *h*, are equipped with silver-silverchloride tips and can be arranged to make contacts directly with the heart. In some instances they served as leads for taking records of the electrocardiogram, and in other experiments they were used as electrodes for exciting a quiescent ventricle preparation.

The bomb, or compression chamber, is completely filled with Ringer's solution and the head, with the manometer and heart attached, is tightly screwed to the bomb while special care is exercised to have the chamber free of air. It is then mounted in a horizontal position on a fairly rigid stand. The small reservoir, *a'*, figure 1, *C*, is essential to the system, since a slight leak in the compression chamber would allow gas to enter it, and thereby materially alter the gas tension immediately surrounding the heart. This reservoir is approximately 100 cc. capacity, and by means of a tube leading from its bottom end, it can be connected to the tube, *a*, on the compression chamber.

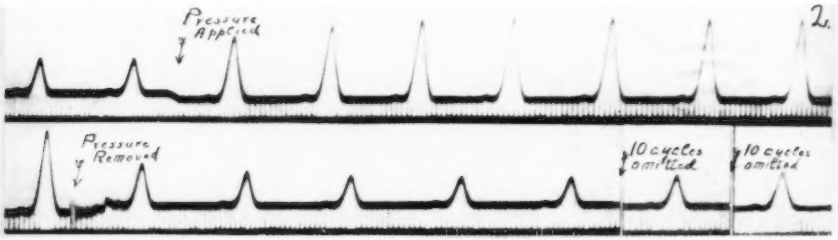


Fig. 2. Changes in the contractility and the rate in the heart of the frog resulting from a compression of 60 atmospheres. The lower record is a direct continuation of the upper record. The last two cycles show recovery.

A large high pressure nitrogen gas tank serves as the source of pressure and this is connected to the compression chamber in the manner shown in figure 1, *C*. The needle valve, *b'*, attached to the nitrogen tank provides a satisfactory means of admitting the pressure into the system, and the stopcock, *c'*, at the top of the reservoir, affords a quick method for the release of the pressure. It is evident, therefore, that with the reservoir partly filled with Ringer's solution, and the inside of the bomb chamber including the manometer and the chambers of the heart, completely filled, a compression of this liquid will result in a hydrostatic pressure of equal force acting uniformly throughout the heart tissue.

A small arc lamp serves to project a beam of light through the window of the compression chamber, and upon the mirror of the manometer. The heart contractions are recorded by the reflection of this beam of light

into a suitable photokymograph. If the heart chambers and the manometer are free of air there is no appreciable change in the resting position of the system when pressure is turned on or off in the bomb chamber. On the other hand, a small amount of air in the heart or the manometer will cause an immediate displacement of the base line of the system, or a rupture of the membrane when the pressure is introduced into the chamber.

We have usually employed in these experiments a pressure of about 60 atmospheres (882 lbs. per square inch), but in some instances pressures as

TABLE 1

The effects of pressure on the rate and the amplitude of contraction of the frog's heart

EXPERIMENT	AMPLITUDE OF CONTRACTION			DURATION OF HEART CYCLE		
	Before pressure	During pressure	After pressure	Before pressure	During pressure	After pressure
	mm.	*mm.*	*mm.*	*seconds*	*seconds*	*seconds*
1-a	70.0	110.0	32.0			
1-b	43.0	113.0	23.0	2.16	2.14	2.56
2	23.0	34.0	22.0	1.55	1.44	1.60
3-a	18.0	24.0	13.0	1.78	1.66	2.10
3-b	19.0	25.0	12.0	2.20	2.02	2.60
4	55.0	68.0	32.0			
4-a				1.58	1.50	1.80
4-b				2.20	2.10	2.10
5	45.0	74.0		2.24	2.16	2.24
6	13.0	18.0	9.0	2.54	2.16	2.76
8	7.0	8.0	4.5	2.72	2.50	2.96
9-a	6.0	14.0	5.0			
9-b	10.0	27.0	5.0			
10	39.0	85.0		1.06	1.04	1.18
11-a	34.0	103.0	26.0	3.70	3.30	4.02
11-b	14.5	36.0	10.0	3.8	3.40	4.90
11-c				2.8	2.66	3.24
11-d				2.72	2.60	3.12
12-a	8.0	18.5	7.5	1.34	1.28	1.42
12-b	14.5	39.0	13.0	1.62	1.60	2.00
12-c	19.0	47.0	15.0	4.32	3.62	4.40
12-d				3.92	3.58	4.16
Average..	25.77 100%	49.6 192%	15.3 59%	2.46	2.27	2.73

high as 80 atmospheres, and as low as 45 atmospheres were tested. A pressure gauge forms a part of the inlet tubing and serves to indicate at all times the amount of compression acting within the bomb.

Experimental results. The immediate and almost invariable effect of compression on a beating heart is a marked increase in the amplitude of its contractions. When a force of 60 atmospheres is placed on the system containing the heart it produces a distinct augmentation in the height of the

first cycle after the onset of the pressure, but the maximum effect does not appear until usually about the third to the fifth cycle. This action of pressure on contractility is shown typically in figure 2. The delay in attaining the maximum effect, as indicated by the successive increase in the amplitude of the cycles 1 to 4 of the pressure series (fig. 2), suggested a possible relation to the manner of introducing the pressure. In the early tests a period of 5 to 10 seconds was commonly necessary to raise the pressure up to the desired amount, but more careful attention to this detail provided a means of raising it to the required 55 to 60 atmospheres in less than one second. In this manner we have been able to show quite conclusively that the influence of pressure on contraction is immediate but its maximum effect is not instantaneous.

In seventeen experiments listed in table 1, the average of the recorded height of the contractions at the time of the maximum effect from compression, shows an increase of 92 per cent over the average of the amplitude of the pre-pressure series. These results are based on measurements of the

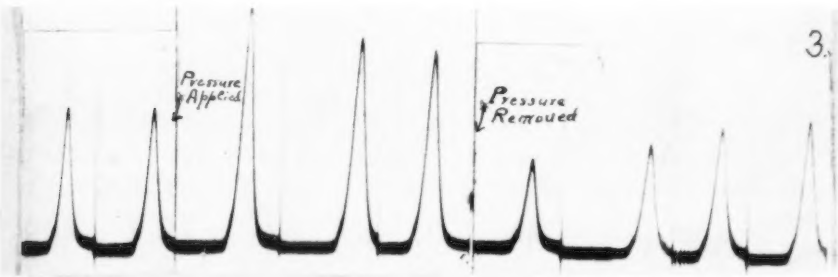


Fig. 3. Effect of a pressure of 60 atmospheres on the contractility of a quiescent ventricle, excited at one minute intervals.

height of contraction in millimeters. A more significant measurement would have been the tension developed, but technical difficulties in the way of calibrating the manometer while enclosed in the compression chamber, made this method impracticable. In individual experiments we have often observed an increase in the contractile response of over 100 per cent, therefore, the average presented in the table is probably low. It represents the total displacement produced by systole of the heart, and this may have been limited in many instances by complete emptying of the ventricle. The diastolic volume of the ventricle remains practically unchanged by compression, therefore, the augmentation in contraction amplitude shown in the records, must be due to a more complete emptying during systole. Moreover, the contractile force developed by the heart is greater, since it acts against an elastic membrane which offers additional resistance in proportion to the increase in the height of the contraction.

The increase in the contraction amplitude under pressure is not, however, an enduring effect as shown by our results. We have maintained the compression on the heart in some experiments for a period of five minutes while the heart continues to beat rhythmically but with gradually diminishing amplitude. It was suggested that this failure to maintain a higher level of activity under pressure might be due to the more rapid using up of energy materials as a result of the stimulating action of compression. In order to test this hypothesis we carried out a short series of experiments using hearts with the sinus ligated so that the ventricle remained quiescent. At one minute intervals an induction shock was given and the resulting contractions of the ventricle recorded. A group of typical contractions of a frog's ventricle is shown in figure 3, before the pressure is applied, and then for three successive minutes while the heart is under a pressure of 60 atmospheres. The results of these experiments show that the principal augmenting effect of compression occurs within the first minute interval, then a conspicuous falling off during the second minute period, and a more gradual decline during successive intervals of one minute. The significant fact brought out in these results is that a heart does not tend to maintain a higher level of contractile function under continued compression.

Release from compression produces a sudden decrease in the size of the contractions that is almost as marked as the augmenting effect with pressure. Table 1 shows that the average height of contraction after the removal of pressure is only 59 per cent of the pre-pressure value. Decrease in the height of the contractions with the termination of compression is always abrupt and is often complete, although in some instances, as in figure 3, a slight additional decline in height is shown in the first two or three cycles after the pressure is removed. The amplitude of the contractions very soon begins to show an increase in height, and in a period usually of 4 to 5 minutes the recovery is practically complete, as shown in the last two recorded cycles in figures 2 and 3.

The foregoing results clearly indicate that the degree of compression used in these experiments has the effect of producing larger and more forcible contractions of the heart. This deduction is at variance with certain of our conceptions of the influence of pressure on the function of tissue; accordingly, a large number of control tests has been necessary to establish this conclusion satisfactorily. One possible source of error was the bulging of the head of the compression chamber, thereby causing it to become more convex and to act somewhat as a lens. We find that there is constantly a slight bulging of the head with pressure giving rise to a small change in the base line as shown in figure 2 at the place designated *pressure applied*. This alteration in form, however, does not produce an appreciable distortion in the size of the manometer movements, as shown by substituting for the manometer a simple electromagnetic device that is

capable of giving excursions of known amplitude under conditions of pressure, and no-pressure. Another question more perplexing in character is the possible change in the properties of the elastic membrane of the manometer when placed under a high degree of compression. We have not been able to solve this matter in a direct way, but indirectly it is eliminated as a probable source of error in our results, we believe, by having demonstrated that skeletal muscle gives a similar type of response under compression when acting upon a torsion wire isometric lever which is likewise enclosed in the compression chamber.

The experimental results show an additional feature of interest in the increase in heart rhythm resulting from compression. In figure 2, the cycle time before the application of pressure is 3.9 seconds; at the time of maximum amplitude under pressure it shortens to 3.6 seconds, and with the removal of pressure the rate slows to a cycle time of 4.2 seconds. In

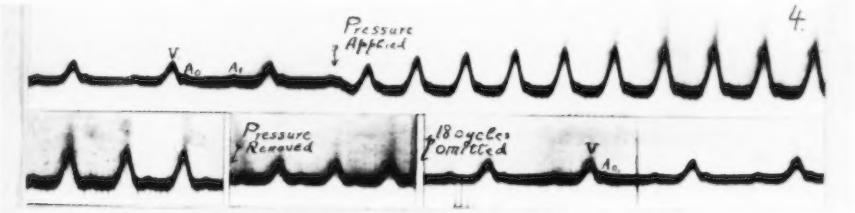


Fig. 4. Action of pressure in removing partial block in a frog heart

eighteen experiments listed in table 1, the average duration of a heart cycle at the outset is 2.45 seconds, and immediately after pressure is applied the average cycle time shortens to 2.27 seconds, which represents an increase of approximately 8 per cent in the rate. When the pressure is removed the opposite change occurs, and the cycle time lengthens to 2.73 seconds which is comparable to a decrease of about 11 per cent in the heart rate.

The changes in rate and in amplitude of contraction displayed by the heart under compression, tend to follow in a similar directional course, and both are manifestations, we believe, of a primary influence of the compression on the excitability of the heart. But there is evidence in each instance of some adaptation to pressure for when the state of compression is maintained for several minutes the height of the contractions declines and the rate slows.

Evidence of an influence of compression on conductivity is given in the results from preparations which exhibited at the outset of the experiments a regular 2:1 type of partial heart block. The tracing shown in figure 4 demonstrates in a conclusive way that pressure applied to this heart facilitated conductivity. After this preparation had been attached to the

manometer and mounted in the usual way, it was shown to be beating rhythmically two auricular to one ventricular contraction, as shown in the record by the isolated auricular wave *Ao* at the foot of the ventricular wave *V*. At the place indicated on the record a pressure of 60 atmospheres is applied and immediately the ventricular rate is doubled, showing that the partial block has been made to disappear. The regular rhythm continues while the pressure is maintained, but with the removal of compression there is at first the usual decrease in amplitude and slowed rhythm, then after a sequence of eighteen beats the 2:1 type of rhythm reappears. Repeated tests on this heart showed a similar type of change in conduction accompanying compression.

These results support the view that compression has a facilitating influence on the transmission of the impulse from auricle to ventricle. There appears to be no *a priori* reason why conductivity within the ventricle should not be influenced in a similar manner, but our attempts thus far to

Fig. 5. The influence of pressure on the response of the isolated heart of the dogfish, excited at one minute intervals.

demonstrate this electrocardiographically have not yielded conclusive results. Certain technical difficulties, which we hope presently to overcome, have entered in our observations on this aspect of the influence of compression on the functions of the heart.

The foregoing results have been obtained entirely from observations on the heart of the frog, but we wish to record at this time the fact that essentially similar responses are obtained with the turtle, and with the dogfish heart. Figure 5 shows a record of the contractions taken at one minute intervals from the isolated ventricle of the heart of a dogfish. It demonstrates that compression has the effect of immediately increasing the contractility of the heart of this species. Moreover, there is indirect evidence in this record of a facilitating effect of pressure on conductivity in the ventricle. Figure 5*A* shows that ventricular action in this heart occurs as a twofold pressure event, with a short delay between wave *a* and *b* of the tracing. This asynchrony in the response of the ventricle is due, we believe, to the slow rate of conduction between parts. In record *B*, taken at the end of one minute under compression at 70 atmospheres, the secondary wave has

partially disappeared, and in *C* little evidence is seen of the secondary wave since it has been moved up closer to the primary wave through the more rapid spread of the impulse in the ventricle. When the pressure is removed the conduction again becomes slowed, and in tracing *F*, the wave *b* is separated by a longer interval from the primary contraction event of the ventricle.

Discussion. The feature of primary interest in these experiments is the evident stimulating effect exhibited by cardiac muscle under a hydrostatic compression of about 60 atmospheres. This type of change is not the commonly predicted action of pressure on the functional properties of a tissue. Especial interest centers in the phenomenon, therefore, from the standpoint of: first, relating it to previous observations on the effects of differential pressure; and second, the mechanism whereby compression causes an apparent increase in excitability.

The depressing action of localized pressure upon the function of muscle and nerve appears to be due to the differential effects at the junctional areas between the compressed and the non-compressed parts. The deformation that occurs at the edges of a compressed region must involve a displacement of the liquid substance of the tissue system and some tissue injury through rupturing of its fiber elements. These changes are sufficient, we believe, to account for the blocking of conduction by pressures of $1\frac{1}{2}$ to 4 atmospheres applied to small areas. On the other hand, extreme compression of 200 atmospheres and above, has an injurious effect as shown by Ebbecke (1914), but the time factor appears to be an important part of this action, since he has shown that the injurious effect of 200 atmospheres acting for a long time is similar to that of 800 atmospheres acting for a short period.

The mechanism of the stimulating effect of grades of compression between 45 and 80 atmospheres acting throughout a tissue must be related, we believe, to changes of a fundamental character in the physical system of the muscular machine. The data at hand do not permit of an adequate analysis of this subject, but experiments are in progress designed to throw more light on this matter. However, it is of interest in this connection to consider that pressure, according to the studies of Fanjung (1894) and of Tammann (1895), causes an increased dissociation of weak electrolyte solutions, and Bridgman (1920) working with a large number of conductive substances, concludes that high compression produces a decrease in the property of electrical resistance. These deductions on the influence of pressure relate to purely physical systems: accordingly they are not to be applied to physiological systems without reservations, but assuming that compression tends to increase the dissociation of the weak acids and bases of the heart, a question of prime importance concerns the possible bearing of such changes upon the phenomenon of the augmenting action of pressure applied to this organ. In this relation it is especially interesting,

therefore, to observe that the experiments of Mayer (1916) show that the rate of nerve conduction bears a close relation to the concentration of ions in the medium, and the observations of Pond (1921) indicate that the velocity of the contraction wave in skeletal muscle is a function of the electrical conductivity of the liquid medium surrounding the tissue. Changes of this nature in muscle under compression may account for the evidences we have obtained of improved conductivity in the rhythmically beating heart. Factors that produce a change in conductivity in muscle produce also an alteration in the same direction in contract[illegible], since both appear to depend upon the state of irritability of the tissue. In this sense, therefore, the contractility of the heart is improved under compression by an increase in the primary condition of excitability.

A change in pressure on a liquid system containing dissolved oxygen has the effect of altering the absolute concentration of this substance. Since the amount of pressure used in our experiments is sufficient to cause a small decrease in the volume of the medium, it follows that the oxygen tension in the environment of the cells must be proportionally increased, and a question arises as to the relation between high oxygen pressure and the functional activity of a tissue. It is not possible to consider this problem fully at the present time, but mention should be made here of the evidence indicating that contraction is an anerobic process, which suggests that the immediate oxygen tension will have little influence on the magnitude of the contractions. Similarly the observations of Lillie (1921) on conduction in the iron wire model, support the view by analogy, that transmission of the excitatory wave in nerve is not a function of the oxygen concentration, except in so far as an adequate supply of this substance is necessary for recovery after conduction.

The work of Gasser and Hill (1924) concerning the rôle of viscosity in muscular contraction, directs attention to this factor as a possible basis of some of the changes in the contractility of the heart under compression. The evidence presented by the work of Bridgman (1925) and others, indicates quite conclusively that liquid systems when subjected to pressure exhibit, with one exception, an increase in viscosity. An alteration of this character reasonably may be expected to occur in muscle under high grades of compression, but present conceptions relating to muscular mechanics make it difficult to understand how a change in the direction of an increase in molecular aggregation, or molecular interlocking, can be correlated with an increase in the amplitude of contraction. On the other hand, pressure has the effect at temperatures below 25°C. of decreasing the viscosity of water, and to some extent also, weak sodium chloride solutions. Since both of these substances enter into the composition of muscle the possibility arises that the effect of pressure on the viscosity of muscle is not a simple one.

The recent observations of Ernst (1926) showing that muscle contraction involves a decrease in volume are of interest in this connection. It follows that hydrostatic pressure, employed according to our technic, exerts an additive effect in the contractile phase, but a reverse action during the relaxation phase. The latter phase, however, involves no active processes by the heart, and under the conditions of our experiments it is normally accomplished by the active recoil of the elastic membrane of the manometer. The resulting effect of compression, therefore, is to augment contractility in a mechanical manner. It is possible also, that pressure exerts a facilitating action upon contraction through an effect on the chemical processes concerned, since uniform pressure tends to further those reactions which are accompanied by a decrease in volume.

A question of considerable interest relative to the volume alteration in muscle accompanying compression, is the shrinkage in muscular mass that apparently must result from the amount of pressure used in our experiments. A pressure of 60 atmospheres on muscle will produce[2] a shrinkage in its volume of approximately 0.3 to 0.4 per cent. This artificial decrease in the volume of the muscle substance tends to diminish the length of the muscle fibers without producing a corresponding change in the initial tension on the fibers. The fact that the heart under these circumstances regularly exhibits a greater contractile response is especially interesting in view of the rôles assigned to initial tension, and initial length of the fiber in cardiodynamics. Our results, however, do not warrant the conclusion that initial length of the fiber plays no part in determining the size of the contractile response, since the fundamental stimulating effect which occurs under pressure, as we have shown in other ways, completely counteracts and obscures any tendency to diminished response that may be present from a decrease in the fiber length. We have made some observations on hearts with different degrees of initial tension in order to test its influence on the amount of augmentation produced by compression. The results lend no support to the view that a greater initial dilatation of the heart promotes an increased amplitude of response under compression.

SUMMARY

A description is given of an apparatus for the application of hydrostatic pressure on all surfaces of the heart. It provides a means also of photographically recording, at any time, the contractions of this organ.

The heart preparations were from the frog, the turtle, and the dogfish, and the results obtained from each species were essentially similar. A pressure of 60 atmospheres was employed in most of the experiments.

Heart preparations with normal rhythm, when placed under compres-

[2] Assuming that muscle has the same compressibility as water.

sion, show an immediate increase in the amplitude of the contractions. A similar effect is shown by ventricular preparations that are stimulated to contract at one minute intervals. In seventeen experiments the average change in the amplitude of contraction at the time of the maximum effect is an increase of 92 per cent over the average height shown before compression. The removal of pressure has the opposite effect on contractility. In sixteen experiments the average height of contraction immediately following the release of pressure shows a decrease of 41 per cent below the pre-pressure amplitude.

There are changes in the rate of the heart with the introduction and removal of compression simultaneously with the changes in the amplitude of the contraction. In eighteen experiments the rate immediately following the onset of pressure shows an increase of 8 per cent, and immediately after the removal of the pressure, a decrease of 11 per cent below the pre-pressure value.

Heart preparations with an established 2:1 block may show an immediate return to normal rhythm when put under compression, indicating that pressure has a facilitating effect on conduction. With release of the pressure the heart block returns.

The application of pressure uniformly to all surfaces of a heart is accompanied by evidences of a stimulation of the organ. The mechanism of this action is discussed in the light of possible alterations in ionic dissociation, and changes in the viscosity of the muscle.

BIBLIOGRAPHY

BRIDGMAN, P. W. 1920. Proc. Natl. Acad. Sci., vi, 505.
1925. Ibid., xi, 603.
CATTELL, McK. AND D. J. EDWARDS. 1926. This Journal, lxxx, 427.
CHLOPIN, G. W. AND G. TAMMANN. 1903. Zeitschr. f. Hyg., xlv, 171.
EBBECKE, U. 1914. Pflüger's Arch., clvii, 79.
ERNST, E. 1926. Ibid., ccxiv, 240.
FANJUNG. 1894. Zeitschr. physical Chem., xiv, 673.
FRÉDÉRICQ, H. 1913. Arch. intern. de physiol., xiii, 311.
GASSER, H. S. AND A. V. HILL. 1924. Proc. Roy. Soc., B. xcvi, 398.
GAERTNER, G. 1920. Pflüger's Arch., clxxx, 90.
HITE, B. H. AND W. MORSE. 1920. Proc. Soc. Exper. Biol. and Med., xvii, 132.
LILLIE, R. S. 1920–21. Journ. Gen. Physiol., iii, 129.
MAYER, A. G. 1915–16. This Journal, xxxix, 375.
POND, S. E. 1920–21. Journ. Gen. Physiol., iii, 807.
REGNARD, P. 1891. Le vie dans les eaux, Paris.
TSCHERMAK, A. 1902. Pflüger's Arch., xci, 217.
TAMMANN, G. 1895. Zeitschr. physical Chem., xvii, 725.
v. UEXKULL. 1895. Zeitschr. f. Biol., xxxi, 153.